Serotype Distribution among *Streptococcus pneumoniae* Isolates in Puerto Rico

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Objective: *S. pneumoniae* infections remain a significant cause of morbidity and mortality despite vaccine availability. Limited information exists about current pneumococcal serotypes transmission in Puerto Rico. Our study aimed to describe the serotype distribution of *S. pneumoniae* isolates in Puerto Rico and its patients' demographic characteristics.

Methods: This prospective laboratory-based observational study from 25 hospitals in Puerto Rico (April 2021-July 2023) collected specimens positive for *S. pneumoniae* and serotyped them using the *Pneumotest-Latex* assay and the *Quellung* reaction tests. A summary of the distribution of S. pneumoniae isolates is presented.

Results: Nineteen specimens were received from sterile (8/19, 42.1%), and non-sterile sites (11/19, 57.9%). All sterile specimens were isolated from blood samples. Most specimens came from male patients (16/19, 84.2%), the median age was 67 years (range: 8 months to 87 years) and came from different geographic regions. Thirteen serotypes were identified: 3 (two patients), 6A (two patients), 11C, 11D, 15A, 15C, 19A (two patients), 19B, 19C (two patients), 19F (two patients), 22A, 34 (two patients), and 35. Of these, nine (69.2%) were not covered in PPSV23 and PCV13 (available vaccines being used prior and by the time of specimen collection period), three (23%) were covered by both PPSV23, and PCV13, and one (8%) by PCV13 only.

Conclusion: Our findings highlight the importance of continuous surveillance to detect early serotype changes, ongoing vaccination to avoid preventable infections and complications, and pursuing new higher-valency vaccines with broad serotype coverage to address the evolving pneumococcal disease epidemiology.

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Key words: Invasive Pneumococcal Disease, Non-invasive Pneumococcal Disease, Streptococcus pneumoniae, S. pneumoniae, Puerto Rico

Pneumococcal disease (PD) caused by *Streptococcus pneumoniae* (*S. pneumoniae*) is a serious public health problem worldwide. In 2017, data using a variety of population-based studies estimated that more than 31,000 cases and 3,500 deaths from invasive pneumococcal diseases (IPD) occurred in the Unites States (1). Other estimates using data from the World Health Organization and the proportion of cases covered by *S. Pneumoniae* identified that between 8 – 12% of all deaths in children were related to this infection (2). However, since the introduction of the pneumococcal conjugated vaccines in children, IPD caused by covered serotypes significantly declined (2).

S. pneumoniae is a gram-positive encapsulated diplococcus that resides in the nasopharynx and is capable of infecting sites contiguous with the upper respiratory tract (e.g. ears, sinuses, lungs) or spreading to distant tissues through the bloodstream (3, 4). The most common manifestations of IPD are bacteremia, pneumonia, sepsis, and meningitis. The most common noninvasive manifestations are sinusitis, acute otitis media and community-acquired pneumonia. Case fatality rates from IPD are highest in young children and older adults. (5,6). Vaccination remains the most effective strategy to reduce the burden of PD. Although pneumococcal vaccines have been proven to be safe and effective in preventing PD, their true benefits depend on the prevailing serotypes in individual countries. Monitoring the epidemiology of PD especially the current serotype distribution, can inform vaccination programs and policies.

As of 2020, more than 100 serotypes have been isolated. However, updated local data on circulating pneumococcal serotypes is limited in Puerto Rico. In 2005 a manuscript (7) was published focusing on the distribution of *S. pneumoniae* in Puerto Rico. It presented the results of a hospital surveillance study for invasive pneumococcal disease conducted in 2001.They found

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that most of the isolated serotypes found were represented in the existing vaccines (78%). As of today, there is no other reported surveillance study in Puerto Rico.

This study aims to improve our understanding of the epidemiology of pneumococcal disease in Puerto Rico, specifically determining the serotype distribution of *S. pneumoniae* isolates in children and adults from 2020 to 2023. This surveillance study will provide valuable insights for the local healthcare system and help us assess the impact of currently used vaccines on the epidemiology of S. pneumoniae in Puerto Rico.

Methods

Study design

This is a non-interventional, prospective laboratory-based observational study in which samples from positive *S. pneumoniae* infected subjects were collected from each participating hospital's microbiology laboratory. Samples were collected as part of the standard of care clinical management of the hospitals. The participating hospitals provided written consent to properly store and send culture-confirmed *S. pneumoniae* samples to a central microbiology lab for serotype testing. Isolates of *S. pneumoniae* were obtained from samples collected from both normally sterile sites (such as blood, cerebrospinal fluid, or joint, pleural, or pericardial fluid) and non-sterile sites (such as the nose or ear). Demographic information, including age, sex, and municipality, along with sample details, was collected by the hospital's laboratory technician and recorded on a de-identified and pseudonymized sample collection form.

Hospital selection

Private and public hospitals in Puerto Rico were invited to participate. Twenty-five hospitals agreed to submit samples, representing all healthcare geographic regions in Puerto Rico. The samples submitted for serotyping were obtained from the cultures of locally collected samples by the microbiology laboratories at the participating hospitals. These samples were collected as per the standard of care and not for the purposes of this study. No additional diagnostic or monitoring procedures were carried out at the participating hospitals.

Inclusion criteria

To be eligible for the study, samples from hospitalized patients must meet all inclusion criteria including: 1) isolated from a *S. pneumoniae* positive culture from normally sterile or non-sterile sites, 2) collected from children older than 6 weeks of age and adults and, 3) collected from patients receiving inpatient or outpatient services including those evaluated at the emergency department or any other ward. The inclusion criteria were reviewed by the investigator or qualified designee to ensure that the sample qualified for the study.

The protocol was approved by the University of Puerto Rico Medical Sciences Campus IRB (Protocol A3490220).

Study variables

Basic demographic information paired with the corresponding pseudonymized de-identified sample was obtained from the hospital laboratory. No personal identifiers or other clinical information was received with the specimens and informed consent was not required in this study. The following sociodemographic and clinical information accompanied the sample: age at the time of sample collection, sex, municipality of residence, clinical site of sample collection, and COVID-19 status. These variables were stratified for analysis purposes including: age group (from 6 weeks old to < 3 years old, from 3 years to < 18 years old, from 18 years to < 65 years and 65 years or more), sex (male or female), site of sample collection (sterile source (e.g. blood, cerebrospinal fluid or joint, pleural, pericardial fluid) or non-sterile source (e.g. nose, ear, sputum) and COVID-19 Status (positive or negative based on reported information).

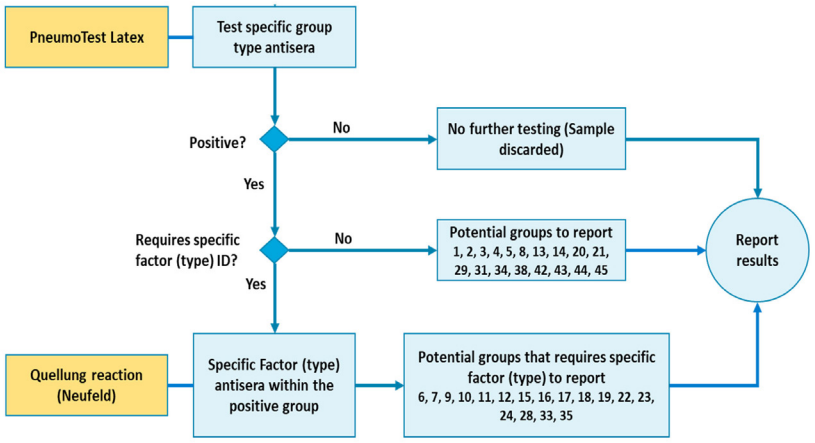
Sample processing

Samples from patients admitted to participating hospitals for routine diagnosis and treatment of suspected *S. pneumoniae* infection were included following hospital laboratory confirmation of *S. pneumoniae* isolates and confirmation of study inclusion / exclusion criteria. Pneumococcus confirmation was performed in each hospital laboratory. An independent central laboratory performed the pneumococcal serotyping using *Pneumotest-Latex* (8-10) and *Quellung* test (11) (SSI Diagnostica, Denmark). The samples were initially typed using the *Pneumotest-Latex* test. This test is a version of a latex agglutination test which uses latex particles coated with rabbit anticapsular antisera. The Pneumotest-Latex test permits rapid testing of culture broth of pneumococcal isolates. The test has been validated elsewhere and is considered today a practical method (9,10). The limitation of the *Pneumotest-Latex* assay is that the test can fully detect only S. pneumoniae serogroups and cannot distinguish between certain specific types (e.g., 9A and 9V or 6A and 6B). Only information about the serogroup or a pool of specific serogroups will be obtained (10). Each sample was initially tested for the interpretation of the serogroup from the agglutination reactions with the 14 latex reagents by using the chessboard system (9-10). As mentioned previously, samples resulting in positive test for serotypes without groups (i.e. 1, 2, 3, 4, 5, 8, 13, 14, 20, 21, 29, 31, 34, 38, 42, 43, 44, and 45 respectively), were not considered for additional serotyping. As of today, there are no different subtypes within these groups that have been identified. Therefore, the remaining positive samples were subjected to further testing using the capsule swelling test (*Quellung* reaction) (11). Figure 1 shows a summary of the laboratory procedures for serotyping.

Specimen and data handling and validation

Participating hospitals reported the isolation of a *S. pneumoniae* sample to the designated personnel for the central laboratory. Once the *S. pneumoniae* positive samples were identified and inclusion and exclusion criteria were verified the specimen was transferred from the hospital microbiology laboratory to the central laboratory. The study related patient information and individual sample was pseudonymized by hospital designated personnel (12). The central laboratory performed the serotyping testing analysis following protocol, and the results along with the relevant information from the patient were transferred to the study investigators.

Figure 1. Summary of laboratory procedures for *S. Pneumoniae* serotyping



Statistical analysis

Descriptive statistics were performed including measures of central tendency (mean, median) and dispersion (standard deviation and 95% CI of the mean, minimum, maximum) for continuous variables and frequency distributions (number, percentage) for categorical variables. All enrolment samples were included in the final analysis. The frequency and percentage of isolates within each serotype, as well as the number and percentage of serotypes included in the PCV13 and PPSV23 vaccines and the non-vaccine serotypes was calculated. Due to the small size of the sample, only descriptive analyses were performed and no evaluation of statistical differences between the groups was evaluated. All the statistical analyses were done using STATA® version 14.2 or higher (STATA Corp., College Station, Texas, USA) (13).

Results

Hospital recruitment and Sample submission

A total of 25 hospitals located in all healthcare regions in Puerto Rico were recruited for sample submission. Figure 2 shows the geographic distribution of participating sites. The mean time of participation for the hospitals was 2.33 years (SD± 0.48). The first specimen was collected in April 2021 and from then to July 2023, we received a total of 19 specimens of *S. pneumoniae* for serotyping. Table 1 summarizes the participating hospitals and the number of specimens submitted. The isolates were received from sterile (8/19, 42.1%), and non-sterile sites (11/19, 57.9%). All sterile samples were isolated from blood. Non-sterile samples included isolates from lower respiratory tract (4/11, 36.4%), sputum (4/11, 36.4%), nose (2/11, 18.2%) and ear secretions (1/11, 9.1%).

Sociodemographic description of patients with *S. pneumoniae* infection

Most specimens came from patients that were males (16, 84.2%). The median age of those patients was 67 years, with a range from 8 months to 87 years and were residents of different healthcare regions, being San Juan (3/19, 15.8%), Bayamón (3/19, 15.8%), and Carolina (2/19, 10.5%) the most frequent municipalities, all from the Metropolitan Area. Figure 2 shows the geographic distribution of the municipality of residence of patients whose samples were submitted for evaluation.

Serotypes identified and selected characteristics

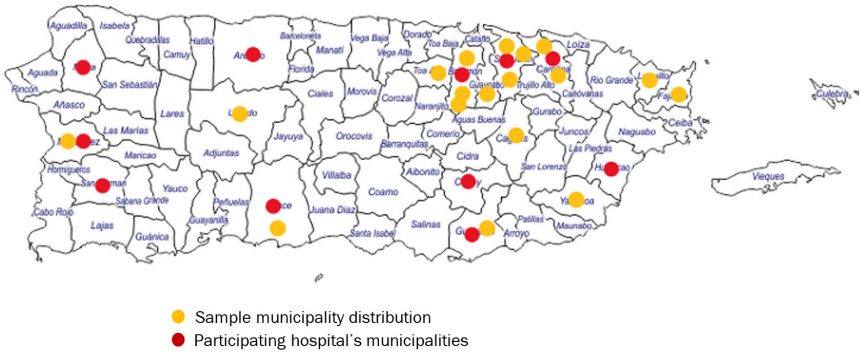
The following 13 serotypes were identified: 3, 6A, 11C, 11D, 15A, 15C, 19A, 19B, 19C, 19F, 22A, 34, and 35. Of these, 9 (69.2%) were not covered in vaccines being used (PCV13, PPSV23) and four serotypes (30.5%) were covered in existing vaccines (3, 6A, 19A, and 19F). Table 1 shows the identified serotypes along with their association with vaccines. No geographical cluster was found for any serotype. Most of the patients were infected with *S. pneumoniae* serotypes that were not covered by vaccines in use by the time of samples collection (11/19, 57.9%). Eight patients (42.1%) had an infection with *S. pneumoniae* serotypes included in PCV13 (2/8, 25%) or both PCV13/PPSV23 (6/8, 75%). Table 2 shows the serotypes identified for sterile and non-sterile sites by age group. Most infections for non-covered serotypes occurred in persons 65 years or older. Of the 19 samples received four (21.1%) came from patients with concomitant COVID-19 positive test. The specimens submitted by these patients were from blood (two patients), sputum, and nose.

Discussion

Our study aimed to evaluate the serotype distribution of *S. pneumoniae* isolated at hospitals settings in Puerto Rico. We found evidence of circulating *S. pneumoniae* serotypes in different geographical regions of Puerto Rico associated with both invasive and noninvasive diseases. Most serotypes identified in sterile and non-sterile sites were not covered by existing vaccines at the time of the study (PCV13 and PPSV23) and occurred in persons older than 65 years. However, all serotypes associated with invasive disease were covered in existing vaccines, highlighting the importance of continuing serotype distribution surveillance and vaccination efforts to prevent these infections and its associated complications.

Several factors could be related to our main findings. In Puerto Rico, the vaccination rate during childhood remains relatively high, (14-16) although there are still important challenges for the vaccination of the adult population. The PCV13 and PPSV23 vaccines were licensed for use in US and Puerto Rico at the time of specimen collection period. (17,18). The widespread use of PCV13 in childhood immunization programs has been associated with a significant decline in vaccine-type IPD, not only in the vaccine target population (i.e., infants and young children), but also among unvaccinated older age groups through herd immunity (19 – 23). At the same time, the emergence of non-vaccine serotypes

Figure 2. Geographical distribution of municipalities of residence of patients with *S. pneumoniae* infection



has been documented in the US and several European countries through serotype replacement effects and are responsible for an increasing proportion of PD (20 – 23). Non-vaccine serotypes may differ in their potential to cause serious clinical outcomes. Certain *S. pneumoniae* serotypes have been linked to more invasive diseases (e.g., meningitis and sepsis) and higher case fatality rates (24).

Several studies in the post-PCV13 introduction have described the pneumococcal serotype distribution among regions or countries. Generally, serotype prevalence can change as a function of time and geographic regions or age groups. One study, evaluating articles from March 2014 to March 2015, highlighted the heterogeneous nature of the serotype distribution (25). The study showed that among children with IPD under the age of seven years the most prevalent serotypes not covered by PCVs were 15B, 22F, 15A, 23A. Among adults with IPD aged ≥ 65 years old serotypes 22F, 11A, 10A, 38 were more prevalent. Likewise, the most prevalent serotypes in adults with IPD aged 50 to 64 years and 15 to 59 years were 12F, 9N, 8 and 12F, 8, 6C, and 16F, respectively (26). None of the previously reported serotypes were found in our study. In another study, a meta-analysis was performed to identify studies and surveillance reports between 2000 and 2015 of pneumococcal serotypes, causing childhood IPD post-PCV introduction (27). They identified 68 studies reporting serotype data among IPD cases in children. They analyzed data from 38 studies (14 countries) where PCV7 was administered and 20 (24 countries) where PCV10 or PCV13 have been introduced. Reductions of IPD associated with vaccine serotypes among young children particularly in North America, Europe, and the Western Pacific were described. Other findings included that about half of childhood IPD cases were due to serotypes for which there is no protection via immunization. Researchers mentioned that serotypes which likely will provide further reductions in disease are 22F, 33F, 15B, 38, and 35B (25% combined) in North America. In addition, serotypes 12F, 22F, 24F, and 33F have been identified to have high invasive disease potential (28). In children, PCVs have been shown to prevent nasopharyngeal carriage of vaccine-type pneumococcal strains and protection against mucosal diseases, IPD, and otitis media. In adults, studies have shown that the efficacy of PPSV23 and PCV13 against PD was comparable (19). Most serotypes identified in our study were previously found in 2001 (7), however, serotypes 11C, 11D, 19B, 22A, and 34 were not previously described.

In this study, we also identified serotypes associated with both invasive and non-invasive infections. This is consistent with the findings previously reported. As example, a study done in Iran to evaluate the serotype distribution among carriers and *S. pneumonia* infection identified the serotypes 19A, 6, 3, 23F associated with both invasive and non-invasive infections (29). In another study carried out in Indonesia in 2018-2019 to evaluate the serotype distribution and antimicrobial profile de *S. pneumonia* infections from patients with community acquired pneumonia, the authors reported that two isolates causing bacteremia were the same serotypes that were isolated from sputum samples (serotypes 6A and 7F) (30).

Just after the beginning of our study, the COVID-19 pandemic was declared. (31-35) Despite intensive surveillance and follow-up with participating hospitals, the number of specimens collected was significantly lower than the number of specimens expected when planning this study. This finding could be related to several factors

Table1. Serotype distribution by vaccine coverage

Serotype	Number of patients	Vaccines in use at the time of samples collection		Higher Valency Vaccines		
		PPSV23	PCV13	PCV15	PCV20	V116
3	2	Y	Y	Y	Y	Y
6A	2	-	Y	Y	Y	Y
11C	1	-	-	-	-	-
11D	1	-	-	-	-	-
15A	1	-	-	-	-	Y
15C	1	-	-	-	-	Y
19A	2	Y	Y	Y	Y	Y
19B	1	-	-	-	-	-
19C	2	-	-	-	-	-
19F	2	Y	Y	Y	Y	-
22A	1	-	-	-	-	-
34	2	-	-	-	-	-
35	1	-	-	-	-	-

Table 2. Serotype distribution in sterile and non-sterile sites isolates by age groups

Serotypes isolated from sterile sites by age group (n=8)				
Age group (years)	**Number of isolates**	**Percent**	**Serotypes included in the vaccine coverage at the time of data collection (PPSV23, PCV13)**	**Serotypes not included in vaccine coverage at the time of data collection**
< 7	1	12.5	19F	-
8 – 14	0	0	-	-
15 – 49	0	0	-	-
50 – 64	1	12.5	3	-
65 or older	6	75.0	6A	11D, 19C, 35,15A, 15C
Serotypes isolated from non-sterile sites serotypes by age group (n=11)				
Age group (years)	**Number of isolates**	**Percent**	**Serotypes included in vaccine coverage at the time of data collection (PPSV23, PCV13)**	**Serotypes not included in vaccine coverage at the time of data collection**
< 14	0	0	-	-
15 – 49	5	45.5	19A, 19F	11C, 22A, 19C
50 – 64	2	18.2	3	34
65 or older	4	36.3	19A (2 isolates)	19B, 34

including potential changes in disease transmission associated with the changing epidemiology of respiratory diseases during and after the COVID-19 pandemic. Most of the period of specimen collection in our study occurred during the time when measures to reduce the impact of COVID-19 were in place, including social distancing, lockdowns, increased hand hygiene and the required use of masks. Since *S. pneumoniae* infections are mainly transmitted by close contact, the implementation of these measures for the control of COVID-19 could result in a concomitant reduction of other respiratory diseases. This phenomenon has been described by other researchers. Brueggemann et al. (36) conducted a prospective analysis of the changes in the incidence of invasive disease due to *S. pneumoniae*, *Haemophilus influenzae*, and *Neisseria meningitidis* during the COVID-19 pandemic in 26 countries and territories using data from the Invasive Respiratory Infection Surveillance Initiative. This was a prospective analysis of surveillance data from laboratories in 26 countries and territories on six continents submitting data on cases of invasive disease due to *S. pneumoniae*, *H. influenzae*, and *N. meningitidis* from January 1, 2018, to May 31, 2020. They found that the introduction of COVID-19 containment policies and public information campaigns likely reduced transmission of those infections, leading to a significant reduction in life-threatening invasive diseases in many countries worldwide. Other studies reported reductions in hospitalizations for non-COVID19 respiratory diseases. Huh K, et al (37) conducted a study finding a decrease in hospital admissions for respiratory diseases during the COVID-19 pandemic. In this study, the authors compared the incidence of hospitalization due to acute respiratory infections (pneumonia and influenza) and chronic respiratory diseases (COPD and asthma) before and during the COVID-19 pandemic. They found that the weekly incidence of hospital admission per 1,000,000 population significantly decreased in almost all diagnoses, being those diseases related to respiratory conditions associated with the most significant decreases in hospitalizations. The reduction in invasive PD and other respiratory pathogens have been reported during the pandemic in other countries. As an example, a study of trend analysis conducted in Japan, comparing IPD data from 2014 to 2019 and during the COVID-19 pandemic from 2020 to 2022 a group of researchers used found a significant decrease in invasive pneumococcal disease during the onset of COVID-19 and between 2019 and 2022 (38). The mechanisms associated with this reduction during the pandemic could be multifactorial and include measures aimed at reducing COVID-19 transmission or potential changes in the circulation of other respiratory pathogens.

Despite the limitations associated with carrying out this study during the COVID-19 pandemic, we were able to identify the circulation of serotypes not previously identified and the presence of serotypes included and not included in pneumococcal vaccines. Our findings highlight the importance of continuous monitoring the circulation of *S. pneumoniae* serotypes in Puerto Rico to allow early identification of serotype replacement, as well as the need to monitor the future impact of the introduction of recently approved higher valency vaccines PCV15, PCV20 and V116.

To those serotypes included in PCV13, PCV 15 added serotypes 22F and 33F while PCV20 added serotypes 22F, 33F, 8, 10A, 11A, 12F and 15B. V116 was developed to address PD in adults and contains 8 serotypes not included in any currently licensed vaccine (15A, 15C, 16F, 23A, 23B, 24F, 31, and 35B). Further studies of public health interventions will contribute to address the evolving impact of PD in our populations.

Resumen

Objetivo: Las infecciones por *S. pneumoniae* son una causa importante de morbilidad y mortalidad. Existe poca información sobre la transmisión de serotipos de neumococo en Puerto Rico. Este estudio describe la distribución de los serotipos de *S. pneumoniae* en Puerto Rico y las características demográficas de sus pacientes. Métodos: Este estudio observacional-prospectivo basado en laboratorio realizado en 25 hospitales de Puerto Rico (abril 2021-julio 2023) recolectó muestras positivas para *S. pneumoniae* e identificó su serotipo utilizando las pruebas "*Pneumotest-Latex*" y "*Reacción de Quellung*". Un resumen de la distribución de los serotipos es presentada. Resultados: Se recibieron diecinueve aislados de muestras estériles (8/19,42.1%) y no estériles (11/19,57.9%). Todas las muestras estériles fueron de sangre. La mayoría de las muestras eran de hombres (16/19,84.2%), la mediana de edad fue de 67 años (rango: 8 meses a 87 años) y provenian de diferentes regiones geográficas. Se identificaron los siguientes trece serotipos de *S. pneumonia*: 3

(dos pacientes), 6A (dos pacientes), 11C, 11D, 15A, 15C, 19A (dos pacientes), 19B, 19C (dos pacientes), 19F (dos pacientes), 22A, 34 (dos pacientes) y 35. De estos, nueve serotipos (69.2%) no están cubiertos por las vacunas PPSV23 y PCV13 disponibles durante el período de recolección de muestras, tres (23.0%) están cubiertos por las vacunas PPSV23 y la PCV13, y uno (8.0%) solo por la vacuna PCV13. Conclusión: Nuestros hallazgos resaltan la importancia de vigilancia continua para detectar cambios en los serotipos, de continuar los esfuerzos de vacunación y de desarrollar nuevas vacunas que aborden los potenciales cambios en la epidemiología de la enfermedad neumocócica.

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