**ORIGINAL ARTICLE**

**Oropharyngeal carriage of Streptococcus pneumoniae by children attending day care centers in Taubaté, SP: correlation between serotypes and the conjugated heptavalent pneumococcal vaccine**

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**Abstract**

**Objective:** *Streptococcus pneumoniae* usually colonizes the oropharynx of healthy people. Oropharyngeal carriage is related to the invasion of adjacent structures and to the development of invasive disease. A descriptive cross-sectional study was performed aiming at verifying the prevalence of oropharyngeal colonization by *S. pneumoniae* in children attending day care centers in the city of Taubaté - SP; verifying the frequency of *S. pneumoniae* serotypes in isolated strains; and relating the most frequent serotypes to the composition of the conjugated heptavalent pneumococcal vaccine currently in use.

**Methods:** from June 29 to December 15 1998, samples of oropharyngeal material were collected from 987 children, ranging from 8 to 71 months old, enrolled in day care centers in Taubaté - SP. The identification of *S. pneumoniae* was based on the observation of the colonies that presented partial hemolysis in agar-blood and agar-blood with gentamicin plates and on the observation of inhibited growth around the optochin disc. Serotyping was performed by the Quellung reaction, using specific antiserum, provided by the Centers for Disease Control and Prevention (Atlanta, GA/USA), and the Danish nomenclature system.

**Results:** as a result, *S. pneumoniae* was isolated from the oropharynx in 209 out of 987 children (colonization rate of 21.2%). Twenty eight serotypes were identified. The seven most frequent serotypes were: 6A/6B (21.5%), 19F (14.8%), 18C (7.4%), 23F (7.4%), 9V (6.7%), 14 (5.2%), 10A (4.4%). Except for serotype 10A, all the other six are included in the vaccine. The only vaccine serotype which was not found was serotype 4.

**Conclusion:** agreement of 63% between the oropharynx colonizer serotypes and the serotypes present in the vaccine was found.


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**Introduction**

For more than a hundred years *Streptococcus pneumoniae* (pneumococcus) have been known as an important causal agent of morbidity and mortality in individuals of ages.1 It is an agent which habitually colonizes the oropharynx of healthy people, considered as asymptomatic carriers. From this state of colonization it is transmitted from person to person in droplets.2 This
asymptomatic carrier state is theoretically related to the appearance of the invasive disease, since there is invasion of adjacent structures by \textit{S. pneumoniae}, such as the middle ear, the paranasal sinuses, the meninges and the bloodstream.\textsuperscript{3,4} The epidemiological study of invasive pneumococcal disease is intimately related to the identification of pneumococcus which colonized the upper respiratory tract. With the appearance of bacterial strains resistant to more usual antibiotics, routine vaccination becomes ever more important.

The prevalence of pneumococcal disease is greatest during the first two years of life,\textsuperscript{5,6} an age at which the polysaccharide pneumococcal vaccine is not effective. The conjugation of capsular polysaccharide antigens with proteins may improve the immunogenicity of the pneumococcal vaccine in children less than two years old, by permitting a T-dependent immunological response.\textsuperscript{5,6}

One obstacle to this proposal is the existence of 90 serotypes of \textit{S. pneumoniae}. It does not appear to be possible to include a large number of antigens in a conjugated vaccine which signifies that vaccinated individuals remain susceptible to serotypes that are not included in the vaccine.\textsuperscript{6}

The conjugated pneumococcal vaccine currently licensed in the USA is composed of the saccharides of the capsular antigens of the serotypes 4, 6B, 9V, 14, 18C, 19F and 23F of \textit{S. pneumoniae} individually conjugated to the diphtheria CRM\textsubscript{197} Protein.

The current study took as its objectives: to verify the general prevalence of oropharynx colonization by \textit{S. pneumoniae} in children attending day care centers in the municipality of Taubaté-SP, to verify frequency \textit{Streptococcus pneumoniae} serotypes in isolated strains, and to relate the most frequent serotypes with the serotypes present in the composition of the heptavalent conjugated pneumococcal vaccine currently in use.

\section*{Methods}

The universal inclusion of all children less than six years old and registered at municipal day care centers in the town of Taubaté was decided upon. The parent or guardian of each child to be included in the study was informed of the research methodology and objectives by the researcher and gave their consent by means of filling out and signing a Universidade de Taubaté standardized informed consent form.

Samples were collected by a researcher pre-trained by the Special Microbiology Unit of the Medical Investigation Laboratory of the Instituto de Medicina Tropical, using a sterile swab which was introduced by the mouth until it touched the oropharynx.\textsuperscript{7} The swab was then conditioned in a transport medium (Amies with charcoal), which permits the bacteria to remain viable at STP for 24 hours, and sent to the Special Microbiology Unit of the Medical Investigation Laboratory (LIM 54) of the Instituto de Medicina Tropical - FM/USP, on average within 4 hours of collection. At the point of collection the children had not eaten for at least 2 hours.\textsuperscript{7} There was no clinical assessment of the children being studied.

The samples were processed by the Microbiology Unit of the Medical Investigation Laboratory (LIM 54) of the FM/USP. They were sown in culture media: agar-blood (AB) and agar-blood with gentamycin (AB-G). The slides were incubated at 35\textdegree C in a 5\% CO\textsubscript{2} atmosphere for 48 hours.\textsuperscript{8}

The identification of \textit{S. pneumoniae} was based on an observation of colonies presenting partial hemolysis in the AB and AB-G media and by an observation of inhibited growth around the optochin disc (5 µg disc).\textsuperscript{7}

The samples were sent to the Bacteriological Support Laboratory of the Medical Microbiology Department of the Instituto de Microbiologia at the Universidade Federal do Rio de Janeiro (IM-UFRJ) for serotyping. The \textit{Quellung} reaction was performed using specific antiserum supplied by the, Centers for Disease Control and Prevention, Atlanta, GA/USA and the Danish nomenclature system.

\section*{Results}

\subsection*{Population studied}

Oropharynx secretion samples were obtained from 987 children registered at 29 municipal day care centers in the town of Taubaté (SP).

From a total of 1,200 children younger than six registered at the municipal day care centers of Taubaté, 213 children were excluded. The excluded children were those whose parents or guardians did not authorize the collection, those who were absent from the day care center on the day of collection and those who had eaten within two hours of the collection.

Ages varied from 8 to 71 months (mean average = 50.6 months; median = 53 months; standard deviation = 9.76). Twenty children were 24 months old or younger, an age at which the immune system is not yet able to deal with the polysaccharide capsule of certain agents, as is the case with the \textit{S. pneumoniae} capsule. The remaining 967 children were more than 24 months old.

Gender distribution was as follows: 498 children (50.5\%) were male. In relation to distribution by race, 812 children (82.3\%) were Caucasian, 98 (9.9\%) of mixed race, 71 (7.2\%) black and six (0.6\%) were Asian (Table 1).

\section*{Prevalence of oropharynx colonization by \textit{S. pneumoniae}}

\textit{Streptococcus pneumoniae} was isolated from the oropharynx of 209 of the 987 children, representing a colonization rate of 21.1\%.
Serotype identification

It was possible to serotype 135 samples of *Streptococcus pneumoniae* (64.59%), since some strains (74 strains - 35.40%) were lost during the storage and transportation processes. Twenty-eight serotypes were found, and are listed in order of frequency: 19F (n = 20; 14.8%), 6A (n = 15; 11.1%), 6B (n = 14; 10.4%), 18C (n = 10; 7.4%), 23F (n = 10; 7.4%), 9V (n = 9; 6.7%), 14 (n = 7; 5.2%), 10A (n = 6; 4.4%), 15B (n = 5; 3.7%), 20 (n = 5; 3.7%), 11A (n = 4; 3%), 22F (n = 4; 3%), 7C (n = 3; 2.2%), 15C (n = 3; 2.2%), 16 (n = 3; 2.2%), 3 (n = 2; 1.5%), 37 (n = 2; 1.5%). The serotypes 5, 13, 15A, 16F, 17F, 19A, 23A, 23B, 24B, 35B and 35F were each observed in only one sample. From two samples (1.5%) *Streptococcus pneumoniae* were isolated which it was not possible to type (Figure 1).

Discussion

The primary objective of this study was to determine the general prevalence of *S. pneumoniae* colonization of children who frequent municipal day care centers in Taubaté, SP. For this reason there was universal inclusion of children less than 6 years old, registered at all of the municipal day care centers in Taubaté. As each day care center serves a district, the sample obtained made possible the evaluation of children who live in all districts of the town.

The population studied was also homogenous in terms of age, sex and race, as is described in Results.

Exclusion criteria were the refusal of parents or guardians to allow participation, non-appearance at the day care center on the day of collection, and having eaten within two hours of the time of sample collection. These inclusion and exclusion criteria permitted a homogenous loss across the various day care centers, and also in terms of age group and sex. Nevertheless, the high level of losses from the studied population (17.7%) must be considered a limitation to the study, which may have underestimated or maybe even overestimated the real colonization prevalence among the population of children attending day care centers in Taubaté.

![Figure 1](image-url) - Frequency of serotypes of *S. pneumoniae* isolated from the oropharynx of children attending the municipal day care centers of Taubaté, SP (n = 135)
The identification of \textit{S. pneumoniae} as a colonizing agent of the oropharynx of an individual is directly dependant upon the methodology used. Results can vary according to the collection technique, the transportation of the material to the laboratory, the culture medium and technique employed and the incubation conditions.

For the performance of this research, oropharynx swabs were used whose collection had been undertaken exclusively by the researcher herself, having been trained in advance. This minimized possible, distortion due to different collection methods. The choice of the oropharynx for swabbing was made due to the less aggressive nature of this method of collection.

There is controversy in published literature over the sensitivity of oropharynx swabs when compared with swabs taken of the nasopharynx. In 1961, Box et al.\cite{9} performed a study of the bacterial flora of the upper respiratory tract in which swabs were compared from the anterior nasal region, the nasopharynx and the oropharynx. It was concluded that in terms of bacterial flora the nasopharynx and the oropharynx are similar and that the material collected from the nasopharynx is similar to that collected from the oropharynx.

In 1997, however, Rapola et al.\cite{10} performed a new study comparing collection techniques when researching into the presence of pneumococcus. There was a 30% positivity level in material collected from the nasopharynx as against 20% positivity for the material from the oropharynx. Yomo et al.,\cite{11} in the same year, determined \textit{S. pneumoniae} colonization both by nasopharynx and oropharynx sampling. The nasopharynx colonization prevalence was 96% and for the oropharynx it was 84%, which demonstrates that the technique of collection from the nasopharynx may be more effective in the detection of asymptomatic carriers of pneumococcus.

In this study, the material was incubated after collection in an appropriate transportation medium (Amies with charcoal), capable of preserving it for 24 hours. The swabs were in fact transported to the microbiology laboratory in a maximum of 4 hours; the time which is necessary to travel from Taubaté (the town where the samples were collected) to São Paulo (where the laboratory is located).

A prevalence of oropharynx colonization by \textit{S. pneumoniae} of 21% was observed and considered low when compared to data in national and international medical literature.

Within Brazil, Ferreira et al.\cite{12} described a prevalence of nasopharynx colonization by \textit{S. pneumoniae} of 34.8%, in children with acute rhinopharyngitis, in the city of São Paulo (SP), between June 1997 and May 1998. Those results denote a greater prevalence than that found in the present study. This fact may be partially explained by the geographical differences, since colonization prevalence varies across different regions depending upon climatic and geographical conditions. In addition, the samples were collected around a year before the samples for the current work and it is known that colonization also varies with time. The variability of colonization prevalence in respect of time and geographical region has been well established by a number of different authors.\cite{13} Furthermore, the method used was collection by swab from the oropharynx. Despite remaining controversial, certain studies suggest that there is a greater colonization prevalence when the nasopharynx is studied.\cite{9,10} Additionally, only children with acute rhinopharyngitis were included which may be considered, by certain authors, to be a risk factor for colonization.\cite{14}

Rey et al.,\cite{15} in a study performed in Fortaleza (CE), in 1998, measured the prevalence of nasopharynx colonization in healthy children attended to at vaccination stations, children at day care centers, and children with pneumonia. In the group of healthy children recruited at vaccination stations, the prevalence of nasopharynx pneumococcus colonization was 49%, while in the group of children from day care centers, the prevalence was much higher (72%). As both groups were sampled by the same technician during the same period it became clear that attendance at a day care center is a risk factor for colonization. Notwithstanding, even taking into consideration the fact that the material was obtained from the nasopharynx, the results of the Rey et al.\cite{15} study show that colonization prevalence in Fortaleza is much greater than that found in Taubaté, making clear the variability of colonization prevalence across different geographical regions.

In Salvador (BA), Ribeiro et al.\cite{16} related that the prevalence of colonization of the nasopharynx by pneumococcus in children less than 6 years old from day care centers was 69.7%, also higher than that found in the current study.

The prevalence of colonization observed in Taubaté was also considered low when compared with data from other countries, which differed among themselves in terms of methodology. Woolfson et al.\cite{17} demonstrated a nasopharynx colonization prevalence of 71.9% among African children with acute rhinopharyngitis who were less than 6 years old. Arnold et al.,\cite{18} found 47% of carriers in the USA, with similar methodology. Dagan et al.,\cite{19} in Israel, found elevated colonization prevalence (63%) among healthy children. Boken et al.\cite{20} described 59% in children between 2 and 24 months old who frequented day care centers in Omaha (USA) in 1995, and 55% in a similar population in Nebraska (USA) in the following year.\cite{21} Of the 209 strains that were isolated it was only possible to serotype 135 samples of \textit{S. pneumoniae} because some strains were lost during the process of storage and transportation. There was, in fact, a 35% loss of \textit{S. pneumoniae} positive samples. This was sufficiently significant to be pointed out as a limitation of the study which might have indicated, for each of the serotypes found, a greater or lesser frequency than that which really occurred among the population. By the same token, serotypes that were considered to have little expression in the current
study may have had that expression minimized by the high percentage of losses.

Twenty-eight of the known 90 serotypes were found. Of the total number of strains, 15 are part of the composition of the 23-valent polysaccharide vaccine and, taking into account immunity crossovers between serotypes, 91.1% of the strains that were isolated can be considered immunopreventable through the use of this vaccine.

The seven most prevalent serotypes in descending order of frequency were, 19F, 6A, 6B, 18C, 23F, 9V and 14. With the exception of the 6A serotype, all of them are included in the heptavalent conjugated vaccine currently available. As serotypes 6A and 6B are genetically related and there is an immunoresponse crossover, it can be stated that the seven most prevalent serotypes in this study are included within the heptavalent conjugated vaccine. Serotype 4 was the only one that is included in the vaccine not to show relevant expression in the current study. Thus it can be estimated that the heptavalent conjugated vaccine currently available offers vaccine coverage for the serotypes most frequently encountered of 63%, very close to the 59.3% estimated by Brandileone et al.,22 in relation to strains which cause invasive diseases.

The most frequent serotype was 19F, found in 14.8% of the samples. This serotype is more frequently found among children.22 This phenomenon occurs because the immunoresponse to different polysaccharide capsular antigens varies with age.23 Serotype 19F is also one of the serotypes most frequently presenting penicillin resistance, perhaps because it is one of the most prevalent among children and consequently one of those most frequently exposed to selective pressure by the continual or repeated use of antibiotics.24

Serotype 6A was the next most frequent (11.1%), followed by serotype 6B (10.4%). These two serotypes are capable of inducing a crossed immunoresponse, i.e. children who develop an immunity to serotype 6A also do so for serotype 6B. This happens because they both share the same α antigen, which is the factor characteristic of an individual type or common to types of the same group. Summing the two, they are responsible for 21.5% of the oropharynx colonizing serotypes of the population studied. These serotypes are also very frequently found in pediatric populations, and resistant to penicillin, in similarity to serotype 19F.22

Serotypes 18C and 23F were the third most prevalent, both isolated in 7.4% of cases. They are also part of the group of those most frequent in children and with greater prevalence of resistance to penicillin and pneumococcal infection by these agents can be prevented by vaccination due to the fact that these serotypes are included in the conjugated vaccine currently.22

Serotype 14 was found in 5.2% of cases. Serotypes 14 and 23F are the serotypes which most frequently present resistance to more than one antibiotic (multiresistant strains).22

Curiously, serotypes 1 and 5, which are highly prevalent in isolations from corporeal fluids from patients with invasive pneumococcal disease in Brazil and in other countries in South America,22,25-29 where not found to an expressive extent in our study. This fact may be explained by regional and time differences or by the fact that certain strains which cause invasive disease (for example serotypes 1 and 5) may be little expressed in colonization.

Conclusions

For the population studied we may conclude that:

- oropharynx colonization prevalence by Streptococcus pneumoniae in children between 8 and 71 months old, who attend municipal day care centers in Taubaté, was 21.1%;
- the seven most prevalent serotypes were: 19F, 6A/6B, 18C, 23F, 9V, 14 and 10A, which correspond to 67.4% of the strains.

A 63% agreement is estimated between the serotypes colonizing the oropharynx and the serotypes which are present in the heptavalent conjugated pneumococcal vaccine currently in use.

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