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Nasopharyngeal carriage of *Streptococcus pneumoniae* in Romanian children before the introduction of the pneumococcal conjugated vaccination into the national immunization programme: a national, multi-centre, cross-sectional observational study

Monica Luminos [a,b], Olga Dorobat [a], George Jugulete [a,b], Gabriel Adrian Popescu [a,b], Dragos Florea [a], Anca Draganescu [a], Anca Streinu Cercel [a,b], Alexandru Rafila [a,b,*] on behalf of the of the SP5000 study group

[a] *National Institute for Infectious Diseases "Prof Dr Matei Bals", Grozovici 1, 021105 Bucharest, Romania*
[b] *University of Medicine and Pharmacy "Carol Davila", Bucharest, Romania*

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SUMMARY

Objectives: We analysed the distribution of vaccine and non-vaccine *Streptococcus pneumoniae* serotypes and the antimicrobial susceptibility of pneumococcal strains isolated from healthy Romanian children.
Methods: A multi-centre cross-sectional study was performed in four counties to evaluate carried strains of *S. pneumoniae* isolated from 2000 children aged 0–5 years.
Results: *S. pneumoniae* carriage was detected in 25.25% of the tested children. Carriage increased from 16.7% among infants to 29.4% in 3–5-year-old children ($p < 0.0001$). The proportions of the serotypes included in pneumococcal conjugate vaccines PCV7, PCV10, and PCV13 among our isolates were 39.9%, 40.1%, and 58.7%, respectively. Erythromycin resistance was 72.5%, and it was significantly lower in non-vaccine serotypes compared with PCV13 serotypes: 57.3% versus 83.6% ($p < 10^{-7}$). Penicillin minimum inhibitory concentrations (MICs) >0.064 mg/l were recorded in 71.6%, but the penicillin MIC was >2 mg/l for only 8.4% of tested isolates.
Conclusions: In Romanian children, the majority of carried *S. pneumoniae* isolates are vaccine serotypes. The isolates with MICs defining macrolide resistance were very frequent, as well as the isolates with MICs defining penicillin resistance in the case of meningitis or penicillin dose-dependent susceptibility for other infections, mainly for the strains belonging to PCV13 serotypes. The implementation of PCV13 within the Romanian national immunization programme could reduce the circulation of these strains with higher macrolide and/or penicillin MICs.

1. Introduction

Pneumococcal conjugate vaccines (PCV) represent a major medical advance, reducing the morbidity and mortality of invasive pneumococcal disease (IPD), community-acquired pneumonia, and acute otitis media caused by serotypes included in these vaccines.[1] However, the successful durability of a national immunization programme may be influenced by the emergence of non-vaccine serotypes after the implementation of vaccination.[2,3]

Streptococcus pneumoniae colonizes the nasopharynx, particularly in young children, where it serves as a reservoir for person-to-person transmission.[4,5]

The distribution of *S. pneumoniae* serotypes is not the same in patients with pneumococcal diseases compared with healthy colonized people, most likely due to the different invasive capacities of the serotypes.[6,7] Studies conducted in different countries indicate a significant decrease in the carriage of vaccine-related strains after the inclusion of PCV in the national immunization programme.[3]

* Corresponding author. Tel.: +40 21 3186100/4128.
E-mail address: arafila@yahoo.com (A. Rafila).

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Antibiotic-resistant *S. pneumoniae* is spreading globally, and epidemiological studies are needed to determine the magnitude of this alarming problem. Most of the available data have analysed the susceptibility of *S. pneumoniae* to two of the most common antibiotics used for pneumococcal diseases: penicillin and erythromycin.[8,9]

PCVs will be introduced for the first time into the routine Romanian childhood immunization schedule in 2014. There is a scarcity of data regarding the *S. pneumoniae* serotypes involved in IPD in different areas of Romania. Moreover, although limited, the information regarding *S. pneumoniae* carriage in children in Romania indicates high antibiotic resistance rates.[10]

Therefore, we designed a national, multi-centre, cross-sectional observational study to assess *S. pneumoniae* carriage and the serotype distribution in healthy children before the large-scale implementation of PCV immunization in Romania. In addition, penicillin and macrolide *S. pneumoniae* susceptibility rates were determined.

2. Methods

2.1. Setting and study design

This prospective, multi-centre, cross-sectional observational study was conducted from November 2011 to April 2013 in four different locations: Bucharest, Iasi, Constanta, and Targu Mures.

Children aged 0–60 months old were enrolled. They were selected from two types of location: paediatric ambulatory care clinics ($n = 500$) and nurseries and school gardens ($n = 1500$); 10 locations in Bucharest and two locations in the other three centres (a maximum of five children included from each class) were selected.

Only immune-competent children were considered eligible. Children with acute respiratory tract infections at the time of enrolment or who had received antibiotic treatment within 10 days of the time of enrolment were excluded. Only one nasopharyngeal swab was taken from each participant. Written informed consent was obtained from a parent/tutor of each child prior to enrolment. Two thousand children were enrolled from 2763 eligible children. The number of tested children from participating centres was 1413 in Bucharest, 217 in Iasi, 167 in Targu Mures, and 203 in Constanta.

The ethics committee of each participating institution approved the study protocol.

2.2. Sampling

A nasopharyngeal sample was obtained using a flexible wire with Dacron/Rayon tip. The swabs were inoculated into MW173 Amies transport medium (Transwab; Medical Wire and Equipment, Potley, UK) and all were processed within 16 h at local clinical microbiology laboratories.

2.3. S. pneumoniae *isolation and identification*

The *S. pneumoniae* isolates were first identified by local microbiology laboratories. For the isolation of *S. pneumoniae*, swabs were inoculated onto Colombia agar with 5% sheep blood and 5.0 μg/ml gentamicin and were incubated aerobically at 35 °C for 48 h. *S. pneumoniae* was identified by colony morphology, α-haemolysis, and inhibition by optochin. The isolates were stored at −20 °C/−80 °C and sent to the central laboratory for confirmation of identification, which was based on haemolysis on agar with 5% sheep blood (Oxoid Ltd, Hants, UK) at 37 °C with 5% CO_2 and growth inhibition by optochin (Oxoid Ltd).

Table 1
Streptococcus pneumoniae carriage rate according to the age group

Age group (*n*)	Carriers, *n* (%)	Carriage rate 95% CI
0–11 months (186)	31 (16.7)	12.0–22.7
12–35 months (751)	162 (21.6)	18.8–24.5
36–60 months (1063)	312 (29.4)	26.7–32.2
Total	505 (25.3)	23.4–27.2

CI, confidence interval.

The susceptibility of *S. pneumoniae* to penicillin and macrolide was determined by European Committee on Antimicrobial Susceptibility Testing (EUCAST) standardized disk diffusion method on Mueller–Hinton agar with 5% blood (Oxoid Ltd) with an erythromycin disk of 15 μg and an oxacillin disk of 10 μg (Oxoid Ltd). If the diameter of the oxacillin inhibition zone was less than 20 mm, penicillin minimum inhibitory concentrations (MICs) were determined by Etest (bioMérieux, Marcy l'Etoile, France). A strain was considered fully susceptible to penicillin when the penicillin MIC was $\leq$0.064 mg/l and resistant if the MIC was $>$2 mg/l. Erythromycin resistance was defined as a growth inhibition zone smaller than 22 mm. Serotyping was performed with type and pooled antisera (Statens Serum Institut, Copenhagen, Denmark) using the chessboard method, in accordance with the manufacturer's recommendations. The tested serotypes were 1, 3, 4, 5, 6, 6A, 6B, 7, 7F, 8, 9, 9 V, 10, 11, 12, 14, 15, 17, 18, 18C, 19, 19A, 19F, 20, 22, 23, and 23F.

2.4. Statistical analysis

We assumed a similar birth rate in the last 6 years to standardize the unequal distribution of patients in the age groups.

The statistical analysis of categorical variables was performed by Chi-square test and Fisher's exact test, using Epi Info 7 software (US Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA). A two-sided *p*-value of $<$0.05 was considered statistically significant.

In the sub-analysis, where carrier rate, serotype distribution, and antibiotic resistance were described by age group, we divided age into three groups: 0–11 months (infancy), 12–35 months (young toddlers), and 36–60 months (older toddlers). The relative risk (RR) and 95% confidence interval (CI) were calculated to estimate the *S. pneumoniae* carriage for each group.

3. Results

Two thousand children were enrolled in the study. The distribution among the three age groups 0–11, 12–35, and 36–60 months was 9.3%, 37.5%, and 53.1%, respectively.

Table 2
Serotype distribution in the three age groups: 0–11, 12–35, and 36–60 months

Serotype	0–11 months ($n = 29$), *n*	12–35 months ($n = 141$), *n*	36–60 months ($n = 283$), *n*	Total ($n = 453$), *n* (%)
6A	3	14	34	51 (11.2)
6B	6	19	35	60 (13.2)
6 non-A, non-B	1	10	26	37 (8.2)
14	1	10	14	25 (5.5)
15	3	12	16	31 (6.8)
19A	4	10	17	31 (6.8)
19F	6	32	54	92 (20.3)
19 non-A, non-F	1	6	26	33 (7.3)
23F	1	5	11	17 (3.8)
23 non-F	0	7	23	30 (6.6)
Other PCV[a]	1	0	4	5 (1.1)
Other non-PCV[b]	2	16	22	39 (8.6)

PCV, pneumococcal conjugate vaccine.
[a] Other PCV serotypes: 9 V, 18C.
[b] Other non-PCV serotypes: 3, 5, 7, 8, 9 non-V, 10, 11, 12, 17, 18 non-C, 20, 22.