

RESEARCH NOTE

Antimicrobial susceptibility and serogroup/serotype distribution of nasopharyngeal isolates of *Streptococcus pneumoniae* in healthy Estonian children in 1999–2003

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ABSTRACT

This study determined nasopharyngeal (NP) carriage rates of *Streptococcus pneumoniae* among healthy Estonian children, aged 1–7 years, and characterised the serotype/serogroup distribution and antibiotic susceptibility rates. NP swabs were collected from 685 previously healthy children attending 29 day care centres during the winters of 1999–2000 and 2003. The NP carriage rate of *S. pneumoniae* was 44%. Rates of penicillin and erythromycin non-susceptibility were low (both 6%), but high (67%) rates of co-trimoxazole resistance were found. Among the pneumococcal serotypes identified, 64% were included in or cross-reacted with the licensed heptavalent pneumococcal vaccine.

Keywords Antibiotic susceptibility, Estonia, nasopharyngeal carriage, penicillin-non-susceptible, serotypes, *Streptococcus pneumoniae*

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Limited data exist concerning the distribution of penicillin-non-susceptible *Streptococcus pneumoniae* (PNSP) in countries of the former Soviet Union. In Russia, despite erratic use of antibiotics in the past, the rate of PNSP has remained at <10%, but high levels of erythromycin resistance have been reported in some areas [1,2]. A low rate of PNSP was also revealed by a previous study conducted in Estonia during the late 1990s [3]. The aim of the present study was to determine the nasopharyngeal (NP) carriage rate, antibiotic susceptibility patterns and serotype distribution of *S. pneumoniae* among children in Estonia, and to identify the risk-factors associated with the carriage of non-susceptible strains of *S. pneumoniae* before the introduction of pneumococcal immunisation.

The study was conducted in three Estonian towns (Tallinn, Tartu and Jõhvi/Kohtla-Järve) during the winters of 1999–2000 and 2003. NP swabs were collected from 685 healthy children, aged 1–7 years, who attended 29 different day care centres. Parents or guardians completed a questionnaire seeking information concerning medical history and antibiotic treatment during the previous 6 months. The study was approved by the Ethical Committee of Tartu University.

Specimens were collected perinasally, using a sterile swab with a flexible aluminium shaft and a dry calcium alginate tip (Eurotubo; Industrias Aulabor, Barcelona, Spain), and were sent to the microbiology laboratory in Stuart transport medium within 8 h of collection. All swabs were then cultured on selective sheep blood agar containing gentamicin 5 mg/L for 24 h at 37°C in CO₂ 5% v/v. *S. pneumoniae* was identified on the basis of colony morphology, α -haemolysis and optochin inhibition. Isolates were stored at –80°C for future serotyping.

Susceptibility to trimethoprim–sulphamethoxazole (co-trimoxazole), tetracycline, rifampicin and clindamycin was determined by disk-diffusion using Mueller–Hinton agar containing sheep blood 5% v/v. Susceptibility to penicillin was determined by disk-diffusion using 1- μ g oxacillin disks. MICs of penicillin were determined by the Etest method (AB Biodisk, Solna, Sweden). Susceptibility to erythromycin during the first study period was determined by disk-diffusion, and during the second study period by Etest. The results were interpreted according to CLSI guidelines [4]. Isolates showing resistance to three or more antibiotics were considered to be

multiresistant. Serotyping of *S. pneumoniae* was performed using the Quellung reaction with Pneumotest kit sera (Statens Serum Institut, Copenhagen, Denmark), according to the manufacturer's instructions.

Sales data for all antimicrobial agents prescribed for children aged <4 years during 2001–2002 were obtained from the central database of the Estonian Health Insurance Fund. Defined daily doses/1000 subjects/day were calculated as described previously [5].

Univariate analysis was used to identify the potential risk-factors for colonisation with *S. pneumoniae*, with the chi-square or Fisher's exact test used for categorical variables, as appropriate. The variables that were found to be significant in univariate analysis were studied by logistic regression. All statistical tests were performed

using the SPSS statistical package (SPSS Inc., Chicago, IL, USA).

In total, 685 children, aged 1–7 years, were recruited to the study (Table 1), with *S. pneumoniae* being isolated from the NP cultures of 299 (44%) children. The NP colonisation rate for all pneumococci was highest for children aged 1–2 years (66%) and declined with age; no age group was associated with carriage of PNSP. The mean penicillin MIC was 0.012 mg/L. Antimicrobial resistance was expressed by 220 (74%) isolates; of these, 12 (4%) were considered to be multi-resistant. Resistance to trimethoprim–sulphamethoxazole (67%) was the most common. In total, 6% of all isolates were either PNSP or resistant to erythromycin, but none showed high-level resistance to penicillin.

In total, 280 isolates were serotyped; 259 (93%) belonged to 26 serogroups/serotypes and 21 (8%) were non-typeable. The most frequently observed serogroups/serotypes, in decreasing order, were 19, 23, 6, 15 and 14, which together accounted for 59% of the isolates. Antibiotic consumption rates in different areas were similar (Table 2). The most commonly used antibiotics were penicillins, followed by macrolides and cephalosporins.

According to univariate and multivariate analysis, residence in Tartu and an age <3 years were associated with carriage of *S. pneumoniae* (OR 1.56, 95% CI 1.13–2.17, and OR 2.5, 95% CI 1.46–4.32, respectively). Risk-factors for the carriage of trimethoprim–sulphamethoxazole-resistant *S. pneumoniae* were age <3 years (OR 1.7, 95% CI 1.00–2.92), day care attendance for >1 year (OR 1.78, 95% CI 1.19–2.65), exposure to cigarette smoke at home (OR 1.54, 95% CI 1.08–2.18) and residence in Tartu (OR 1.71, 95% CI 1.20–2.43).

Overall, the study showed that in Estonia, as in other northern European countries and Russia, the prevalence of PNSP from NP samples was low [2,3], but that there were high levels of resistance

Table 1. Characteristics of children recruited to the study

Characteristic	Total number of children n (%)	No. of <i>Streptococcus pneumoniae</i> carriers n (%)
Gender		
Female	339 (49)	148 (44)
Male	346 (51)	151 (44)
Age (years)		
1–3	239 (35)	118 (49)
4–5	301 (44)	124 (41)
6–7	145 (21)	57 (39)
Siblings		
Yes	406 (59)	175 (43)
No	279 (41)	124 (44)
Cigarette smokers at home		
Yes	345 (50)	162 (47)
No	340 (50)	137 (40)
Duration of day care attendance		
0–1 year	257 (38)	132 (51)
>1 year	428 (62)	167 (39)
Otitis media in previous 6 months		
Yes	87 (13)	31 (36)
No	598 (87)	268 (45)
Antibiotics within previous 6 months		
Yes	218 (32)	86 (39)
No	467 (68)	213 (46)
Geographical area		
Tartu	292 (43)	144 (49)
Tallinn	191 (28)	66 (35)
Kohtla-Järve/Jõhvi	202 (29)	89 (44)

Table 2. Antibiotic consumption rate (defined daily doses/1000 children) for children aged <4 years in Estonia during 2001–2002

Study location	No. of children	No. of DDD/1000 children				
		Total	Penicillins	Macrolides	Cephalosporins	TMP-SMX
Tartu	13 115	15.4	7.8	4.5	1.9	1.2
Tallinn	4103	13.3	8.6	2.3	1.5	1.0
Kohtla-Järve/Jõhvi	1513	11.2	4.3	3.0	0.8	1.1

DDD, defined daily doses; TMP-SMX, trimethoprim–sulphamethoxazole (co-trimoxazole).

to trimethoprim–sulphamethoxazole, the drug that was used widely to treat respiratory tract infections in the past [6]. Recently, Goossens *et al.* [7] revealed a significant correlation between penicillin use and pneumococcal resistance rates in a European multi-national study, and found that outpatient antibiotic consumption rates in Estonia were among the lowest in Europe. Furthermore, as in the present study, the use of oral cephalosporins was rare. This suggests that conservative antibiotic prescribing practices are common in outpatient settings, with penicillins being preferred to newer macrolides and oral cephalosporins, and that these two factors may have played a significant role in the low rate of PNSP in Estonia. As in previous studies [8,9], age <3 years and exposure to cigarette smoke from family members were associated with the carriage of trimethoprim–sulphamethoxazole-resistant isolates.

The most common serogroups associated with NP carriage and invasive disease worldwide are 6, 14, 19 and 23, but differences have been observed in their distribution among industrialised and non-industrialised countries [10]. These four serogroups were also commonest in the present study; however, in contrast to other industrialised countries, in which 80–90% of disease-causing strains would be included in or cross-react with the licensed heptavalent vaccines, only 64% of NP isolates would be covered by the vaccine in Estonia [10].

In conclusion, most *S. pneumoniae* isolates in Estonia remain susceptible to penicillin and erythromycin, but high levels of resistance to trimethoprim–sulphamethoxazole were revealed. This indicates that penicillins are still the drugs of choice for treatment of pneumococcal infections, while the use of trimethoprim–sulphamethoxazole for respiratory tract infections should be avoided. Continuous national monitoring of the antimicrobial resistance and serotype distribution of *S. pneumoniae* is essential

for designing immunisation strategies and advising on antibiotic use.

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