Nasopharyngeal carriage and antibacterial susceptibility of *Streptococcus pneumoniae, Haemophilus influenzae* and *Moraxella catarrhalis* in Estonian children

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The most common bacterial infections in children are acute sinusitis and otitis media. Approximately 80% of these infections are caused by *Streptococcus pneumoniae*, *Haemophilus influenzae,* and *Moraxella (Branhamella) catarrhalis* [1,2]. Invasive strains of these bacteria, especially *Haemophilus influenzae* type b (Hib), constitute the most important bacterial cause of meningitis in children and cause a substantial proportion of pneumonia cases in several countries [3]. In most of the developed countries, vaccination has reduced Hib infection and carriage rate [4], but Hib and pneumococcal vaccines have not yet been introduced in Estonia.

The upper respiratory tract of healthy children is frequently colonized with *H. influenzae*, *M. catarrhalis* and *S. pneumoniae* [5,6]. This nasopharyngeal colonization is a prerequisite for the development of sinusitis, otitis media or more serious infections. Nasopharyngeal and invasive strains in the same age group apparently have similar antimicrobial susceptibility profiles [7,8]. Today, the emergence of resistant bacteria such as β-lactamase-producing *M. catarrhalis* and *H. influenza*, and penicillin-resistant *S. pneumoniae*, has complicated the empirical therapy of these infections in several countries [1,2,7,9–13]. Thus, local surveillance of nasopharyngeal carriage of resistant strains is important for initiating adequate empirical antimicrobial therapy.

Unfortunately, there is a lack of data on the carriage rates and resistance patterns of these pathogens in Estonia as well as in most other central and eastern European countries. The aim of our study was to evaluate these in Estonian children.

Children without signs of respiratory infection and having had no prior antibiotic treatment within a 2-week period were eligible for the study. Altogether, 396 children aged 2–7 years (average 4) attending 16 day-care centers from three different cities of Estonia were included: Tartu, South Estonia ($N = 200$); Tallinn, North Estonia ($N = 95$) and Jõhvi, East Estonia ($N = 101$). There were no significant differences in age or sex between children from different cities.

Samples were collected at the end of winter or early spring of 1999 and 2000. Nasopharyngeal swabs were collected and transported to the laboratory within 4 h in Stuart transport medium (Eurotubo, Industrias aulabor, s.a., Barcelona, Spain). Specimens were seeded on chocolate agar for isolation of *M. catarrhalis* and *H. influenzae* strains, and on sheep blood agar supplemented with gentamicin 2 mg/L for *S. pneumoniae* [9]. Plates were incubated overnight at 37 °C in a 7% CO_2 atmosphere. Bacteria were identified by the following tests: optochin (BBL, Cockeysville, MD, USA) and Pneumoslide Test (BBL) for *S. pneumoniae*; X, XV and V factor detection (BBL) for *H. influenzae*; and Kligler Iron agar (Oxoid, Basingstoke, England), Urea agar (Oxoid), SIM Medium (Oxoid), OF Medium (BBL), Oxidase (BBL) and Tributyrin test (A/S Rosco, Taastrup, Denmark) for *M. catarrhalis*.

Antimicrobial susceptibility by the disk diffusion method was performed according to NCCLS recommendations [14] for the following*: S. pneumoniae*—rifampin, trimethoprim–sulfamethoxazole, erythromycin and tetracycline; *H. influenzae*—ampicillin, amoxycillin–clavulanate, trimethoprim–sulfamethoxazole, and cefotaxime; *M. catarrhalis*—ampicillin. Minimal inhibitory concentrations (MICs) of penicillin against *S. pneumonia* strains were determined by E-test (AB Biodisk, Solna, Sweden). Strains with MICs below 0.125 mg/L were considered to be fully susceptible, and those with MICs above 1 mg/L fully resistant. The susceptibility tests were performed on Mueller–Hinton agar (Oxoid) with 5% sheep blood for *S. pneumoniae*, on Mueller–Hinton agar (Oxoid) for *M. catarrhalis*, and on Haemophilus Test Medium (Oxoid) for *H. influenzae* strains. For quality control, standard type strains were included [14].

β-Lactamase production by *H. influenzae* and *M. catarrhalis* was detected using the nitrocefin test (BBL). To detect weak β-lactamase production by *H. influenzae*, MICs were determined for amoxycillin and amoxycillin–clavulanate. For detection of

Table 1 Susceptibility of *S. pneumoniae* (number of strains (%))

	Rifampin	Trimethoprim–sulfamethoxazole	Erythromycin	Tetracycline	Penicillin
Susceptible	181 (99.5)	52 (28.6)	175 (96.2)	120 (65.9)	166 (91.2)
Intermediate	–	17 (9.3)	1 (0.5)	33 (18.2)	16 (8.8)
Resistant	1 (0.5)	113 (62.1)	6 (3.3)	29 (15.9)	–

invasive Hib strains, an agglutination test with type b antiserum was used (Difco, Detroit, MI, USA).

Data were analyzed by a Jandel SigmaStat 2.0 program using χ^2, Fisher and Wilcoxon Signed Rank Tests.

Nasopharyngeal carriage with at least one of the screened pathogens was observed in 313 of 396 (79%) children. Of 396 children, 182 (46%) were colonized with *S. pneumoniae*, 68 (17%) with *H. influenzae* and 63 (16%) with *M. catarrhalis*. In 66 (16%) children, two different pathogens were isolated: *S. pneumoniae* and *H. influenzae* in 42; *S. pneumoniae* and *M. catarrhalis* in 22; and *M. catarrhalis* and *H. influenzae* in two.

MIC values of penicillin against strains of *S. pneumoniae* varied between 0.003 and 1 mg/L ($MIC_{50} = 0.012$ mg/L; $MIC_{90} = 0.065$ mg/L). The susceptibility of *S. pneumoniae* strains is shown in Table 1. Two isolated strains were simultaneously intermediately or fully resistant to penicillin, trimethoprim–sulfamethoxazole, tetracycline and erythromycin, and four strains to penicillin, trimethoprim–sulfamethoxazole and tetracycline. As many as 44 (24%) of *S. pneumoniae* strains were susceptible to all tested antibiotics.

All isolated *H. influenzae* strains were β-lactamase negative and highly susceptible to ampicillin, cefuroxime and cefotaxime. On comparing the MICs of amoxycillin–clavulanate and amoxycillin in *H. influenzae* strains, differences were found to be not significant (within one twofold dilution). Of 68 *H. influenzae* strains, 43 (63%) strains were susceptible, five (7%) intermediate and 20 (30%) resistant to trimethoprim–sulfamethoxazole. *H. influenzae* strains isolated from 20 children (5%) belonged to serotype b.

Of 63 *M. catarrhalis* strains, 62 (98%) were β-lactamase positive and only one (2%) negative. Despite this, most of the strains showed large inhibition zones with ampicillin disks (12–44 mm, median 24).

Children in Tartu (South Estonia) were more frequently colonized with *S. pneumoniae* (52%) than those in Tallinn (North Estonia, 37%; $P = 0.026$). Overall colonization with *M. catarrhalis* and *H. influenzae* in investigated cities was similar, but children in Tartu were more frequently colonized with Hib (8.5%) than those in Jõhvi (East Estonia, 1%; $P = 0.019$), and in Tallinn (2%). Bacteria isolated in Tartu were more resistant: 16% of *S. pneumoniae* strains were intermediately resistant to penicillin in Tartu versus 0% in Tallinn ($P = 0.03$), and 0% in Jõhvi ($P = 0.013$); 77% of *S. pneumoniae* strains were intermediately or fully resistant to trimethoprim–sulfamethoxazole in Tartu versus 49% in Tallinn ($P < 0.001$); 54% of *H. influenzae* strains were intermediately or fully resistant to trimethoprim–sulfamethoxazole in Tartu versus 17% in Tallinn ($P = 0.045$) and 0% in Jõhvi ($P < 0.001$). Similar trends were also present in cases of other bacteria and antibiotic combinations—strains isolated in Tartu as compared to those isolated in Tallinn or Jõhvi were usually more resistant (although statistically non-significantly).

We found that the upper respiratory tract of about 80% of 2–7-year-old children was colonized with potential agents of acute otitis and sinusitis. The most frequently isolated pathogen was *S. pneumoniae*. Similar high colonization rates for *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* were found in some other studies [5,6]. Relatively high rates of Hib carriage were found, especially in the children of Tartu. High carriage of Hib has also been reported in other countries in which vaccination programs have not been introduced [15].

Only 9% of the isolated *S. pneumoniae* strains were intermediately resistant to penicillin, but, surprisingly, none of the strains was fully resistant. This finding differs from some recent studies in other European countries, where 4–50% of isolates were found to be highly resistant to penicillin [1,2,5–7,9–13]. Erythromycin resistance was also relatively low (4%) as compared to other European countries.

Up to 47% of the isolated *H. influenzae* strains have been reported to be β-lactamase producers [9,11,13]. No β-lactamase-positive strains were found in this study. Nitrocefin is, however, only weakly labile to some β-lactamases produced by *H. influenzae* [16]. Because of this, some β-lactamase-positive *H. influenzae* strains may have been missed. For detection of these strains, parallel MICs of amoxycillin and amoxycillin–clavulanate were determined in isolated *H. influenzae* strains. These MICs, however, did not differ by more than one twofold dilution, indicating the absence of β-lactamase production.

The production of β-lactamase among *M. catarrhalis* strains was frequent and similar to that found in other studies. A disk diffusion test on β-lactamase-positive strains failed to show resistance to ampicillin. It is known that if standard inoculum is being used, some β-lactamases that are expressed at a low level cannot be detected [16]. Production of such enzymes can explain the high in vitro sensitivity to ampicillin of most of our β-lactamase-positive strains. Treatment failure with ampicillin

has been reported [16]. Therefore, β-lactamase-producing *M. catarrhalis* strains are considered to be resistant to ampicillin according to the NCCLS [14].

This study shows that, besides the differences from other countries, data from different regions of Estonia varied as well. Pathogens isolated in Tartu (South Estonia) were usually more resistant, and Hib carriage was also higher in children of this region. Possible causes of this tendency, such as differences in antibiotic consumption in different regions, should be evaluated.

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