Seasonal variations in nasopharyngeal carriage of respiratory pathogens in healthy Italian children attending day-care centres or schools

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The aim of this study was to investigate seasonal variations in the prevalence of the nasopharyngeal carriage of respiratory pathogens and identify factors affecting colonisation patterns in healthy children. The nasopharyngeal carriage of *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* during two seasons (autumn and spring) was evaluated in 1580 healthy children aged 1–7 years by means of a cohort study conducted in day-care centres and schools in eight Italian cities. A questionnaire was used to obtain the epidemiological data. In all, 309 children (19.5%) carried one or more respiratory pathogens in the autumn, and 375 children (23.7%) in the spring. This variation was due to *H. influenzae* alone or in combination (autumn: *S. pneumoniae* 60, 3.8%; *H. influenzae* 206, 13.0%, *M. catarrhalis* 71, 4.5%; spring: *S. pneumoniae* 75, 4.7%; *H. influenzae* 288, 18.2%, *M. catarrhalis* 82, 5.2%). Colonisation with two or more pathogens increased from 9.1% in the spring to 17.3% in the autumn. Seasonal variations occur in the prevalence of the nasopharyngeal carriage of respiratory pathogens in healthy children attending day-care centres or schools in Italy. However, although statistically significant, the difference was slight and had limited clinical relevance. Therefore, seasonal influence on the nasopharyngeal carriage of respiratory pathogens in healthy children was negligible.

Introduction

Streptococcus pneumoniae, *Haemophilus influenzae* and *Moraxella catarrhalis* can colonise the nasopharynx and can cause upper respiratory tract infections, including otitis media and sinusitis [1, 2].

The prevalence of nasopharyngeal carriage of the potential pathogens varies according to age, geographic area, crowding, concomitant respiratory tract illness and antibiotic consumption [3–7]. Prospective studies have revealed considerable intra-individual quantitative and qualitative changes in nasopharyngeal flora over time [8–10]. Most studies have involved small populations, evaluated a single pathogen, were carried out in sick children, or primarily enrolled infants and young

children. Variables related to the dynamics of nasopharyngeal carriage or antibiotic use have been studied extensively, but the role of seasonal variation has not been studied in a large population. Knowing the influence of season on the nasopharyngeal carriage of respiratory pathogens might be useful for epidemiological surveys. The present study investigated the influence of season on the prevalence of the nasopharyngeal carriage of respiratory pathogens in healthy Italian children and attempted to identify the factors affecting colonisation patterns.

Patients and methods

Study design

The study was part of the Italian Ascanius Project, which was designed in 1996 to evaluate the nasopharyngeal carriage of respiratory pathogens among healthy children aged 1–7 years attending day-care centres or the first 2 years of primary school [10]. Eighteen cities (10 in northern, 3 in central and 5 in southern Italy)

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†See Appendix.

participated in the study. Children with any underlying illness (including chronic otitis media with effusion), or any even mild acute upper or lower respiratory tract infection in the previous 2 weeks were excluded. The first survey was done in the autumn (mid-October to mid-November), at the beginning of the school season; the same children were then scheduled for nasopharyngeal sampling in the spring (mid-April to mid-May). To be eligible for the second nasopharyngeal culture, they had to be disease-free at the time of sampling.

Written questionnaires concerning demographics and medical history, including the number and type of respiratory infections (rhinitis, otitis media, tonsillitis, sinusitis, pneumonia) and the number and type of antimicrobial drugs administered in the 3 months preceding sampling, were completed by the parent(s) in the presence of a paediatrician. Signed informed consent was obtained from a parent or guardian of each child. Further details of the study protocol were reported in the article describing the results of the first survey [7].

Specimen collection

The nasopharyngeal specimens were obtained by the same specially trained investigator in each centre. A Mini-Culturette® (Becton Dickinson, Cockeysville, MD, USA) extra-thin flexible wire swab, with the tip bent at an angle of *c.* 30° was inserted through the child's mouth and placed 1 to 1.5 inches into the nasopharynx, without touching the uvula or the tongue. The children were not allowed to eat or drink during the 3 h preceding specimen collection.

Microbiological procedures

The same procedures were used by all of the participating centres, with guidelines validated by the chief microbiologist (G.C.S). The nasopharyngeal cultures were inoculated into Stuart (Venturi Transystem, Italy) transport medium tubes, which were taken to the microbiology laboratory of the hospital of each paediatric department involved within 1–3 h of collection and processed immediately. *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* were isolated and identified by standard laboratory procedures. At each site, the isolates were stored in serum-glycerol freezing medium at –80°C; the frozen isolates were then sent to the University of Genoa's Institute of Microbiology to confirm their identity and for antimicrobial susceptibility testing. Identity was confirmed by the following procedures. *H. influenzae*: Gram's stain morphology, X (haemin), V (nicotinamide adenine dinucleotide) and combined XV factor dependency; *S. pneumoniae*: Gram's stain morphology, catalase reaction, optochin susceptibility and bile solubility; *M. catarrhalis*: Gram's stain morphology, catalase reaction and hydrolysis of tributyrin. The MICs of the antimicrobial agents were determined by the 'Sensititre microbroth'

incorporation technique (Trek Diagnostic System Ltd, East Grinstead, W. Sussex) with an inoculum of *c.* 10⁵ cfu/ml. The medium used was Mueller-Hinton broth, supplemented with saponin-lysed horse blood and factor V for isolates of *Haemophilus* spp. and *S. pneumoniae*. The antimicrobial agents tested were penicillin, amoxicillin, amoxicillin/clavulanate, cefaclor, cefuroxime, cefixime, ceftriaxone, erythromycin, clarithromycin and azithromycin. The MIC breakpoints used for the qualitative interpretation of the quantitative data were based on those published by the National Committee for Clinical Laboratory Standards [11].

Data management and analysis

The data were recorded on pre-coded forms and analysed using release 6.12 of the SAS program (SAS Institute, Cary, NC, USA). The quantitative variables were compared by means of Student's test and, if necessary, the qualitative variables by means of the χ^2 test with Yates' correction or Fisher's exact test. All the reported *p* values are two-sided and refer to a significance level of 5%.

Results

Study participation

Table 1 shows the demographic characteristics of the study cohort, which included the 1580 children sampled during the two seasons. Most of the children lived in an urban area, attended a day-care centre full time, and belonged to small families. None of them had received *H. influenzae* b vaccine. There was no significant difference between the children sampled in the autumn and spring in terms of family size, living conditions, full-time attendance at a day-care centre or passive smoking exposure. The cohort consisted of 91.7% of the 1723 children enrolled in the autumn survey because 143 children did not return for the spring sampling session due to the following reasons: moved outside the study area (*n* = 65), changed school (*n* = 25), were unwilling to be sampled again (*n* = 15) or developed a chronic illness (*n* = 38).

Table 1. Characteristics of the study cohort

Characteristic	Number of children (%) = 1580
Male	815 (51.6)
Race (white)	1552 (98.2)
Age (mean)	4.39
1–3 years	611 (38.7)
4–5 years	390 (24.7)
6–7 years	579 (36.6)
Breast-feeding ≥ 3 months	1166 (73.8)
Living in urban area (population: >10 000)	1270 (80.4)
Full-time day-care attendance*	1478 (93.5)
Previous day-care attendance (>1 year)	853 (53.9)
Having older sibling(s)	972 (61.5)
Living in a small family (3–4 people)	1446 (91.5)
Passive smoking	750 (47.5)

* Attendance for 5–6 days/week, 6–8 h/day.

Prevalence of nasopharyngeal carriage of respiratory pathogens in the two seasons

Table 2 shows the nasopharyngeal colonisation of respiratory pathogens in the two seasons. In all, 309 children (19.5%) carried respiratory pathogens in the autumn, compared with 375 (23.7%) in the spring ($p = 0.004$). The proportion of carriers colonised by two or three pathogens increased significantly between the autumn (28 of 309, 9.1%) and spring (65 of 375, 17.3%) ($p = 0.002$).

The carriage of *H. influenzae*, alone or in combination with other pathogens, was significantly more common in the spring than in the autumn ($p = 0.02$ and $p = 0.0001$, respectively). No significant difference in the carriage rates of *S. pneumoniae* or *M. catarrhalis* was found between the two surveys.

Antibiotic susceptibility patterns

The susceptibility patterns of the respiratory pathogens in the two surveys were not significantly different. Among the *S. pneumoniae* isolates, only a few isolates were penicillin resistant – autumn: three isolates (5%), two intermediately resistant and one fully resistant; spring: four isolates (5.3%), three intermediately resistant, one fully resistant – whereas resistance to macrolides was very common – autumn: 25 isolates (41.6%), 1 intermediately resistant, MIC ≥ 0.5 mg/L, 24 fully resistant, MIC ≥ 1 mg/L; spring: 30 isolates (40%), 2 intermediately resistant, 28 fully resistant. All *S. pneumoniae* isolates were susceptible to amoxicillin, independently of the season. Resistance to amoxicillin remained stable for the *M. catarrhalis* isolates – autumn: 63 isolates (88.7%); spring: 73 isolates, 89.1% – and increased slightly and in a non-significant manner in the isolates of *H. influenzae* – autumn: 12 isolates (5.8%); spring: 29 isolates, (10.1%).

Variables associated with differences in nasopharyngeal carriage in the two seasons

Table 3 summarises the association between nasopharyngeal colonisation and respiratory tract infections or

antibiotic courses in the 3 months preceding each sampling session. The overall proportion of children who had experienced respiratory tract infections or had been given antibiotics was greater in the spring than in the autumn, but there was no difference in these variables between the carriers of one or more pathogens and the non-carriers in each study period. The drug types used were similar in both periods.

The proportion of children that had two or more respiratory tract infections in the preceding 3 months was significantly higher in the spring than in the autumn only in the subjects carrying *H. influenzae* – autumn: 31 of 206 (15.0%); spring: 67 of 288 (23.3%); $p = 0.03$ – and children carrying two or more pathogens – autumn: 3 of 28 (10.7%); spring: 22 of 65 (33.8%); $p = 0.04$. No significant difference was found between the autumn and spring among the carriers of *H. influenzae* or multiple pathogens, or in the use or type of antibiotics. No other significant differences were found.

Discussion

The data from the present study seem to indicate that the prevalence of nasopharyngeal carriage of respiratory pathogens can vary over time, with a small but significant increase from autumn to spring, in healthy children attending day-care centres or schools. *H. influenzae*, alone or in combination, was the main organism responsible for this increase. However, although statistically significant, the increase in the proportion of children who were asymptomatic nasopharyngeal carriers of respiratory pathogens between the autumn and spring was slight and of limited clinical relevance.

These findings differ from those of the limited number of reports involving infants and children followed over time. Harvey and Dunlap [3] did not find any consistent seasonal fluctuations in the nasal carriage of *S. pneumoniae*, *H. influenzae* or haemolytic

Table 2. Nasopharyngeal colonisation of respiratory pathogens in the autumn and spring

Carrier status	Number (%) of children (total = 1580)		
	Autumn	Spring	p value
Carriers of one or more pathogens	309 (19.5)	375 (23.7)	0.004
<i>S. pneumoniae</i>	60 (3.8)	75 (4.7)	0.23
<i>H. influenzae</i>	206 (13.0)	288 (18.2)	<0.0001
<i>M. catarrhalis</i>	71 (4.5)	82 (5.2)	0.40
Carriers of one pathogen	281 (17.8)	310 (19.6)	0.20
<i>S. pneumoniae</i>	47 (2.9)	39 (2.5)	0.44
<i>H. influenzae</i>	185 (11.7)	228 (14.4)	0.02
<i>M. catarrhalis</i>	49 (3.1)	43 (2.7)	0.59
Carriers of two pathogens	28 (1.8)	60 (3.8)	0.0008
<i>S. pneumoniae</i> + <i>H. influenzae</i>	6 (0.3)	26 (1.6)	0.0007
<i>S. pneumoniae</i> + <i>M. catarrhalis</i>	7 (0.4)	5 (0.3)	0.77
<i>H. influenzae</i> + <i>M. catarrhalis</i>	15 (0.9)	29 (1.8)	0.04
Carriers of three pathogens	0	5 (0.3)	0.06

Table 3. Association between respiratory tract infections and antibiotic prescriptions in the preceding 3 months and nasopharyngeal colonisation in autumn and spring

Parameter	Number (%) of children					
	Autumn			Spring		
	Total (n = 1580)	Carriers (n = 309)	Non-carriers (n = 1271)	Total (n = 1580)	Carriers (n = 375)	Non-carriers (n = 1205)
Respiratory tract infections:						
none	741 (46.9) [†]	149 (48.2)	592 (46.6)	669 (42.3)	168 (44.8)	501 (41.6)
one	547 (34.6)	100 (32.4)	447 (35.1)	596 (37.8)	137 (36.5)	459 (38.1)
two or more	292 (18.5)	60 (19.4)	232 (18.3)	315 (19.9)	70 (18.7)	245 (20.3)
Antibiotic therapy*	427 (27.0) [‡]	81 (26.2) [§]	346 (27.2)	503 (31.8)	126 (33.6)	377 (31.2)
Aminopenicillins	110 (6.9)	21 (6.8)	89 (7.0)	133 (8.4)	32 (8.5)	101 (8.4)
Amoxicillin-clavulanate	33 (2.1)	4 (1.3)	29 (2.3)	50 (3.2)	12 (3.2)	38 (3.2)
Macrolides	140 (8.8)	28 (9.1)	112 (8.8)	145 (9.2)	37 (9.9)	108 (8.9)
Cephalosporins	142 (8.9)	28 (9.1)	114 (8.9)	175 (11.1)	45 (12.0)	130 (10.8)

*One or more courses of antibiotics.

Autumn versus spring: [†]p = 0.01; [‡]p = 0.003; [§]p = 0.04; ^{||}p = 0.02. The differences between carriers and non-carriers were not significant in either study period.

streptococci in 97 family groups. More recently, Ingvarsson *et al.* [4] observed that the colonisation rate of respiratory pathogens decreased from 69% to 55% in healthy children between November and April, without any difference in the distribution of individual bacteria. Furthermore, increased carriage of *S. pneumoniae* in the winter has been described by Gray *et al.* [12]. Brook and Gober [13] observed an increase in the carriage of β -lactamase-producing bacteria between the autumn and spring in children with acute pharyngotonsillitis.

Possible explanations for the discrepancies between the data from the latter studies and the present study include the following. First, the large size of the present cohort study population was more representative of the enrolled age groups and geographic sites, and may have avoided the risk of focusing on specific situations. Secondly, the longitudinal follow-up of the same cohort assured a better description of seasonal variation than that obtained from random samples (with different populations). Thirdly, the cohort in the present study included only healthy children who, even if attending day-care centres or schools, had rarely experienced upper respiratory tract infection within the 3 months preceding both samplings. This excluded the possible effect of concomitant viral infections or mild underlying diseases such as otitis media with effusion on nasopharyngeal carriage [5, 13], and may also account for the low prevalence of carriage in both sessions. Moreover, in the two observational periods there were no epidemic conditions that might have affected the results.

The present study had two limitations: seasonal variations would have been better described if there were more sampling points or if observations had been repeated in other years, and the lack of biotyping, which would have allowed a better understanding of the dynamics of the individual pathogens. However, the

main purpose of the study was not to assess the dynamics of a specific organism, but to evaluate the influence of season on the prevalence of the nasopharyngeal carriage of respiratory pathogens in healthy children attending child-care centres.

Respiratory tract infections in the preceding 3 months were not associated with the carriage of any of the respiratory pathogens in the autumn [7], but a history of two or more episodes of upper respiratory tract infection was the only variable associated with the higher carriage rate of *H. influenzae* in the spring. As most upper respiratory tract infections occurring in the winter are caused by viruses, the increase in the carriage rate of *H. influenzae* in the spring might be explained on the basis of data derived from in-vitro studies, which showed that respiratory viruses (particularly adenovirus) can enhance bacterial adherence to the nasopharynx and thus influence nasopharyngeal carriage [14, 15].

The susceptibility patterns of the isolates studied did not change significantly over time. The rates of penicillin resistance among *S. pneumoniae* and β -lactamase production among *H. influenzae* isolates were always very low, and consistent with previous Italian reports regarding adults and children with respiratory tract infections [10, 16, 17]. In a child-care centre, Boken *et al.* [18] recently observed a significant reduction from 53% to 7% in penicillin-resistant *S. pneumoniae* colonisation over a 4-month period, concomitant with a marked decrease in antibiotic use. Furthermore, Brook and Gober [13] have suggested that the decrease from winter to summer in the recovery rate of β -lactamase-producing bacteria could be the result of a different exposure to β -lactams in the two seasons. In the present study, the slight increase in antibiotic consumption between autumn and spring may have prevented the seasonal reduction described in other studies, whereas the similar distribution of the

type of drugs used in the 3 months preceding each sampling may have avoided the selective colonisation of resistant isolates.

In conclusion, if the data are analysed with regard to their importance in planning collective surveys of nasopharyngeal carriage in healthy children attending day-care centres or schools [19], the slight increase in carriage from autumn to spring seems to be of limited practical value, and suggests that such surveys can be undertaken without regard to season.

Appendix

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