Nasopharyngeal *vs.* adenoid cultures in children undergoing adenoidectomy: prevalence of bacterial pathogens, their interactions and risk factors

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SUMMARY

Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis and *Staphylococcus aureus* colonization of the adenoids and nasopharynx in 103 preschool children who underwent adenoidectomy for recurrent upper respiratory tract infections was examined. Bacterial interactions and risk factors for bacterial colonization of the nasopharynx and adenoids, separately, were analysed statistically. The prevalence of simultaneous isolation from both anatomical sites was 45·6% for *S. pneumoniae*, 29·1% for *H. influenzae*, 15·5% for *M. catarrhalis* and 18·4% for *S. aureus*. Three pathogens were significantly more frequent together from adenoid samples; nasopharyngeal swabs more often yielded a single organism, but without statistical significance. *M. catarrhalis* and *S. aureus* significantly more frequently co-existed with *S. pneumoniae* and *H. influenzae* than with each other and a positive association of *S. pneumoniae* and *H. influenzae* in adenoid samples was evident. Several differences between risk factors for nasopharyngeal and adenoid colonization by the individual pathogens were observed. We conclude that the adenoids and nasopharynx appear to differ substantially in colonization by pathogenic microbes but occurrence of *H. influenzae* and *S. pneumoniae* in the nasopharynx could be predictive of upper respiratory tract infections.

Key words: Adenoids, bacterial interaction, colonization, nasopharynx, risk factors.

INTRODUCTION

Nasopharyngeal colonization by bacterial pathogens is a primary stage for the development of upper respiratory tract infection (URTI) of young children due to *Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis*, and *Staphylococcus aureus* [1–3]. The well-known risk factors that promote carriage and spread of these pathogens in children include host immunity, overcrowding (e.g. daycare centre attendance), exposure in the family, number of siblings, passive smoking, age, and female gender [4, 5].

The adenoids play a crucial role in the aetiology of otitis media, rhinosinusitis and adenotonsillitis and knowledge of their microbial flora in individual patients is important for their treatment. Several previous studies have explored the range of bacteria colonizing the adenoids and both positive and negative associations between species colonizing the nasopharynx and adenoids have been reported [6–8], but with contradictory results. There are also some data

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Table 1. *Demographic data of children undergoing adenoidectomy for recurrent upper respiratory tract infections*

Risk factor	Total no. of children (%)	Colonized children (% in group)			
		SP	Hinf	Mcat	SA
Age (years)					
2	11 (10·7)	6 (54·5)	8 (72·7)	4 (36·6)	4 (36·4)
3	24 (23·3)	11 (45·8)	19 (79·2)	9 (37·5)	7 (29·2)
4	51 (49·5)	33 (64·7)	36 (70·6)	13 (25·5)	18 (35·3)
5	17 (16·5)	12 (70·6)	10 (58·8)	5 (29·4)	9 (52·9)
Sex					
Female	43 (41·7)	26 (60·5)	31 (72·1)	20 (46·5)	17 (39·5)
Male	60 (58·3)	36 (60·0)	42 (70·0)	11 (18·3)	21 (35·0)
Having siblings					
0	17 (16·5)	11 (64·7)	12 (70·6)	5 (29·4)	7 (41·2)
1	46 (44·6)	28 (60·9)	35 (76·1)	15 (32·6)	12 (26·1)
⩾2	40 (38·8)	23 (57·5)	26 (65·0)	11 (27·5)	19 (47·5)
Passive smoking	32 (31·1)	19 (59·4)	22 (68·8)	10 (31·3)	11 (34·4)
DC attendance	86 (83·5)	49 (59·0)	59 (68·6)	24 (27·9)	34 (39·5)
Place of residence					
Village	41 (39·8)	23 (56·1)	28 (68·3)	16 (39·0)	13 (31·7)
City	62 (60·2)	39 (62·9)	45 (72·6)	15 (24·2)	25 (40·3)
Antibiotics taken for last episode					
Amoxicillin/co-amoxiclav	55 (54·5)	34 (61·8)	45 (79·0)	19 (33·3)	22 (38·6)
Cephalosporins	24 (23·8)	16 (66·7)	11 (45·8)	6 (25·0)	7 (29·2)
Macrolides	22 (21·8)	10 (45·5)	17 (77·3)	6 (27·3)	9 (40·9)

DC, Daycare centre; SP, *Streptococcus pneumoniae*; Hinf, *Haemophilus influenzae*; Mcat, *Moraxella catarrhalis*; SA, *Staphylococcus aureus*.

concerning significance of nasopharyngeal samples to predict acute or recurrent otitis media [9] or to corroborate a diagnosis of pharyngotonsillitis [10] but data correlating nasopharyngeal and adenoid bacterial flora in children with recurrent respiratory tract infections are lacking.

In this study we investigated the differences and correlations in occurrence and associations of the main pathogens colonizing the upper respiratory tract (*S. pneumoniae, H. influenzae, M. catarrhalis* and *S. aureus*) in the nasopharynx and adenoids in preschool children (aged 2–5 years) who underwent adenoidectomy for recurrent URTIs. Risk factors for such colonization were also investigated in this patient cohort.

METHODS

Patients

The study enrolled 103 children, aged between 2 and 5 years, undergoing adenoidectomy in the Department of Paediatric Otolaryngology, Phoniatry and Audiology, Medical University of Lublin, Poland during May–June and November–December 2011. The indication for adenoidectomy was recurrent URTIs for at least 2 years with ⩾5 acute episodes per year. Patients did not receive antibiotic therapy for at least 20 days before the operation. In each case, informed consent was obtained from the parents and the study protocol was approved by the Ethical Committee of the Medical University of Lublin (no. KE-0254/75/211).

Demographic data of the studied children is shown in Table 1. All patients had received antibiotics during the 2–3 months prior to surgery but none had been immunized with a pneumococcal vaccine; 25 children were vaccinated against *H. influenzae* type b.

Laboratory procedures

Before adenoidectomy, under general anaesthesia, nasopharyngeal specimens were obtained through the nostril with sterile alginate-tipped swabs on an aluminium shaft. The surgeon removed the adenoids, under the control of an endoscope, via the mouth by making several small incisions and cauterized the site once the adenoids are removed. Antiseptic and/or

bactericidal agents were not used during the surgery. The adenoids were placed in a sterile container and transported together with the nasopharyngeal swabs within 2 h to the laboratory. One surface of adenoid was cauterized with a heated scalpel and an incision was made through that cauterized area with a sterile scalpel cutting the adenoid in half. The core was swabbed with a sterile alginate-tipped applicator and swabs were inoculated on Mueller–Hinton agar with 5% sheep blood with and without 0·5 mg/l of gentamicin for selective cultivation of pneumococci, *Haemophilus* chocolate agar (bioMérieux, France), mannitol salt agar (bioMérieux) for selective cultivation of *S. aureus*, McConkey agar (Biocorp, Poland) for selective cultivation of Gram-negative rods and Sabourand agar (Biocorp) for selective cultivation of yeasts. The plates were incubated aerobically at 35 °C or in 5% CO_2 enriched atmosphere for 24–48 h. Pneumococci were identified by colony morphology, susceptibility to optochin (5 μg), and bile solubility and confirmed by a slide agglutination test (Slidex Pneumo-kit, bioMérieux). *M. catarrhalis* and *H. influenzae* were identified by macroscopic, microscopic and biochemical assays by API NH microtest (bioMérieux). Isolates of *S. aureus* were identified by colony morphology, biochemical activities (ID32 STAPH, bioMérieux), coagulase test and a slide agglutination test (Slidex Staph-kit, bioMérieux), β-haemolytic streptococci were identified by susceptibility to bacitracin (0·8 U/disc) (bioMérieux) and agglutination test for the grouping of streptococci (Slidex Strepto Plus, bioMérieux). The identification of Gram-negative rod isolates was determined using an oxidase test (Taxo N; Becton, Dickinson, USA) and Api 20E or Api 20NE (bioMérieux) as appropriate. Yeasts were identified by colony morphology, Gram staining and biochemical method (API Candida, bioMérieux).

Definitions

Nasopharyngeal culture was representative for nasopharynx microflora including microflora of adenoid surface. Adenoid culture pertained to microflora of adenoid core.

Statistical analysis

Data processing and analysis were performed using Statistica v. 10 (StatSoft Inc., USA). Potential predictor variables were tested by separate univariate analyses (χ^2 or Fisher's exact test, as appropriate) for bacterial association with upper respiratory colonization. Relative risks (RR) and their 95% confidence intervals (CI) were calculated. Logistic regression models were fitted to identify risk factors associated with bacterial carriage in nasopharynx and adenoids, separately. From these models, adjusted odds ratios (OR) and 95% confidence intervals were derived; corresponding *P* values were from Wald's test. Goodness of fit was checked using Hosmer & Lemeshow's test. Variables of particular interest based on previous studies, such as age, having siblings, passive smoking and type of antibiotic used, were included even when not statistically significant. Statistical significance was set at $P < 0.05$. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated for nasopharyngeal culture in relation to adenoid samples.

RESULTS

Bacterial carriage of targeted pathogens

In total, 103 children aged 2–5 years undergoing adenoidectomy were enrolled in the study (Table 1), and 206 paired nasopharyngeal and adenoid samples were analysed. The overall carriage rates of the targeted pathogens in both anatomical sites were for *S. pneumoniae* (64·1%, $n = 66$), *H. influenzae* (70·9%, $n = 73$), *M. catarrhalis* (30·1%, $n = 31$) and *S. aureus* (36·9%, $n = 38$). In addition, in 11 children group A streptococci were detected (two children were colonized in nasopharynx and adenoids and nine children in adenoids only). In nine children other pathogens were isolated, namely group G streptococci (three isolates from adenoid samples), one isolate each of group C streptococci, *Escherichia coli*, and *Pseudomonas aeruginosa* from adenoid samples, *Acinetobacter baumanii* (one isolate from a nasopharyngeal swab) and *Candida albicans* (two isolates from adenoid samples).

There were statistically significant differences in the frequency of bacterial isolation from both sample sites. *H. influenzae* was the main pathogen recovered from adenoids compared to *S. pneumoniae* [$P = 0.001$, RR 1·5, 95% CI 1·2–1·9], *M. catarrhalis* ($P < 0.0001$, RR 4·1, 95% CI 2·6–6·3) and *S. aureus* ($P < 0.0001$, RR 2·1, 95% CI 1·6–2·9). By contrast, *S. pneumoniae* predominated in nasopharyngeal samples compared to *H. influenzae* ($P < 0.0001$, RR 2·1, 95% CI 1·5–3·0), *M. catarrhalis* ($P < 0.0001$, RR 2·2, 95% CI 1·6–3·1) and *S. aureus* ($P < 0.0001$, RR 2·8, 95% CI 1·9–4·1). *M. catarrhalis* and *S. aureus*, respectively, were more
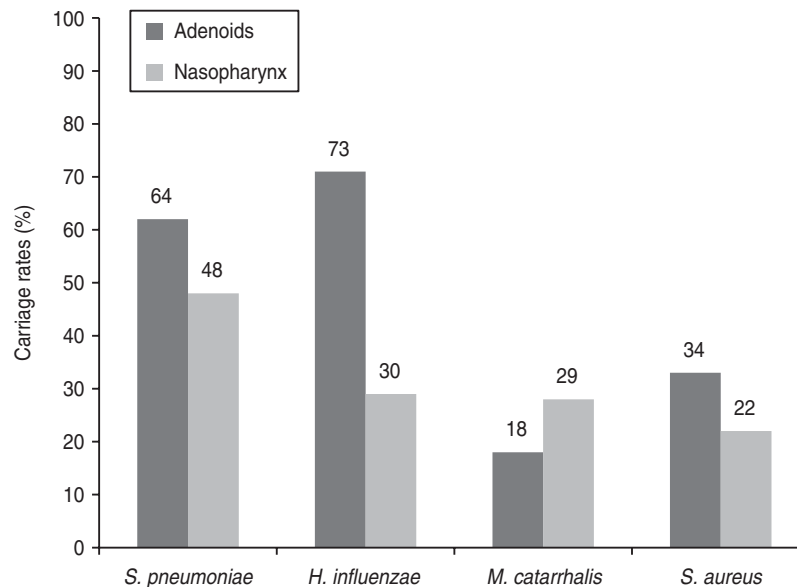
Fig. 1. Bacterial colonization according to place of isolation in children undergoing adenoidectomy for recurrent upper respiratory tract infection. Number of colonized children are shown at the top of the bars.

frequently isolated from nasopharyngeal and adenoid samples (Fig. 1).

The distribution of the targeted pathogens isolated from both anatomical sites and their associations are presented in Table 2. Compared to adenoid samples, nasopharyngeal swabs were significantly less likely to be positive for the target species ($P = 0.0001$). There was a tendency for more frequent isolation of two pathogens together (40·8%, $P = 0.07$), and significantly, three pathogens together from adenoid samples ($P = 0.02$). A single pathogen was most often recovered from nasopharyngeal swabs (39·8%, $P = 0.38$) with *M. catarrhalis* being the most predominant ($P = 0.003$), or together with *S. pneumoniae* ($P = 0.02$). The most frequent co-colonizing species in all samples were *S. pneumoniae* and *H. influenzae* (10·7%) and this combination occurred significantly more frequently in adenoid samples ($P = 0.009$).

Analysis of co-colonization, presented in Table 3, showed positive associations in adenoid samples for concomitance of *S. pneumoniae* and *H. influenzae* ($P < 0.0001$) compared to other species, and *M. catarrhalis* and *S. aureus* more often co-existed with *S. pneumoniae* and *H. influenzae* than with each other. No significant co-colonization was observed for nasopharyngeal swabs.

Correlation between nasopharyngeal and adenoid flora

Coherence of the same species recovered from nasopharyngeal and adenoid samples was found for

S. pneumoniae, H. influenzae, M. catarrhalis and *S. aureus* in 47 (45·6%), 30 (29·1%), 16 (15·5%) and 19 (18·4%) children, respectively. In 17 (16·5%) children *S. pneumoniae* was isolated from adenoid samples only, and in two (1·9%) children it was present in nasopharynx only. *H. influenzae* occurred alone in adenoid samples in 43 (41·7%) children, but was never isolated alone from the nasopharynx. *M. catarrhalis* and *S. aureus* were recovered only from adenoid samples from two (1·9%) and 15 (14·6%) children, respectively, and from the nasopharynx in 13 (12·6%) and four (3·9%) children, respectively.

Analysis of sensitivity, specificity and PPVs and NPVs of nasopharyngeal colonization as indicative of the aetiology of recurrent URTIs for each pathogen showed relatively high specificity for each species (0·85–1·0); sensitivity was moderate (0·73) for *S. pneumoniae*, good (0·89) for *M. catarrhalis* and low for *H. influenzae* (0·41) and *S. aureus* (0·55). The PPVs ranged from 0·55 for *M. catarrhalis* to 0·96 for *S. pneumoniae* and 1·0 for *H. influenzae*, and NPVs from 0·41 for *H. influenzae* to 0·97 for *M. catarrhalis*.

Risk factors and carriage

Several statistically significant factors were associated with predisposition of subjects with nasopharyngeal and adenoid colonization to recurrent URTIs (Table 4). By multivariate analysis, female gender ($P = 0.004$) and city residence ($P = 0.024$) were identified as independent factors contributing to carriage

Table 2. *Distribution of bacteria in adenoids and nasopharynx in children undergoing adenoidectomy for recurrent upper respiratory tract infections*

Bacteria present	No. of patients (%)			
	Adenoids	Nasopharynx	OR (95% CI)	*P*
No pathogens	4 (3·9)	23 (22·3)	0·14 (0·04–0·4)	0·0001*
One pathogen	34 (33·0)	41 (39·8)	0·7 (0·4–1·3)	0·38
Streptococcus pneumoniae	11 (10·7)	16 (15·5)	0·6 (0·2–1·5)	0·4
Haemophilus influenzae	17 (16·5)	8 (7·8)	2·3 (0·9–5·7)	0·08
Moraxella catarrhalis	0 (0)	9 (8·7)	0·04 (0·003–0·8)	0·003*
Staphylococcus aureus	6 (5·8)	8 (7·8)	0·7 (0·2–2·2)	0·78
Two pathogens	42 (40·8)	29 (28·2)	1·8 (1·0–3·1)	0·07
S. pneumoniae + *H. influenzae*	22 (21·4)	8 (7·8)	3·2 (1·3–7·6)	0·009*
S. pneumoniae + *M. catarrhalis*	2 (1·9)	11 (10·7)	0·2 (0·04–0·8)	0·02
S. pneumoniae + *S. aureus*	6 (5·8)	4 (3·9)	1·5 (0·4–5·6)	0·7
H. influenzae + *M. catarrhalis*	2 (1·9)	1 (1·0)	2·0 (0·2–22·6)	1·0
H. influenzae + *S. aureus*	9 (8·7)	4 (3·9)	2·4 (0·7–8·0)	0·2
M. catarrhalis + *S. aureus*	1 (1·0)	1 (1·0)	1·0 (0·06–16·2)	1·5
Three pathogens	21 (20·4)	8 (7·8)	3·0 (1·3–7·3)	0·02*
S. pneumoniae + *H. influenzae* + *M. catarrhalis*	11 (10·7)	4 (3·9)	3·0 (0·9–9·6)	0·1
S. pneumoniae + *H. influenzae* + *S. aureus*	10 (9·7)	3 (2·9)	3·6 (1·0–13·4)	0·08
S. pneumoniae + *M. catarrhalis* + *S. aureus*	0 (0)	1 (1·0)	0·3 (0·01–8·2)	1·0
H. influenzae + *M. catarrhalis* + *S. aureus*	0 (0)	0 (0)	—	—
Four pathogens	2 (1·9)	2 (1·9)	1·0 (0·1–7·2)	1·4

OR, Odds ratio; CI, confidence interval.
* Statistically significant.

Table 3. *Associations between* Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, *and* Staphylococcus aureus *in adenoid and nasopharyngeal samples from children undergoing adenoidectomy for recurrent upper respiratory tract infections*

Bacteria	Co-colonization	No. of adenoid samples (%)			No. of nasopharyngeal samples (%)		
		(*n* = 103)	OR (95% CI)	*P*	(*n* = 103)	OR (95% CI)	*P*
SP	Neither	11 (10·7)	Referred	—	16 (15·5)	Referred	—
	Hinf	45 (43·7)	6·1 (3·1–13·5)	<0·0001*	17 (16·5)	1·0 (0·5–2·3)	1·0
	Mcat	15 (14·6)	1·4 (0·6–3·3)	0·53	18 (17·5)	1·2 (0·6–2·4)	0·85
	SA	18 (17·5)	1·8 (0·8–4·0)	0·23	10 (9·7)	0·6 (0·3–1·4)	0·29
Hinf	Neither	17 (16·5)	Referred	—	8 (7·8)	Referred	—
	SP	45 (43·7)	3·9 (2·0–7·5)	0·0001*	17 (16·5)	2·3 (0·9–5·7)	0·086
	Mcat	15 (14·6)	0·8 (0·4–1·8)	0·85	7 (6·8)	0·9 (0·3–2·5)	1·0
	SA	21 (20·4)	1·3 (0·6–2·6)	0·59	9 (8·7)	1·1 (0·4–3·1)	1·0
Mcat	Neither	0 (0)	Referred	—	9 (8·7)	Referred	—
	SP	15 (14·6)	36·2 (2·1–615·0)	<0·0001*	17 (16·5)	2·1 (0·9–4·9)	0·14
	Hinf	15 (14·6)	36·2 (2·1–615·0)	<0·0001*	7 (6·8)	0·8 (0·3–2·1)	0·80
	SA	3 (2·9)	7·2 (0·4–141·4)	0·25	4 (3·9)	0·4 (0·1–1·4)	0·25
SA	Neither	6 (5·8)	Referred	—	8 (7·8)	Referred	—
	SP	18 (17·5)	3·4 (1·3–9·0)	0·016*	10 (9·7)	1·3 (0·5–3·4)	0·81
	Hinf	21 (20·4)	4·1 (1·6–10·7)	0·003*	9 (8·7)	1·1 (0·4–3·1)	1·0
	Mcat	3 (2·9)	0·5 (0·1–2·0)	0·50	4 (3·9)	0·5 (0·1–1·6)	0·37

OR, Odds ratio; CI, confidence interval; SP, *Streptococcus pneumoniae*; Hinf, *Haemophilus influenzae*; Mcat, *Moraxella catarrhalis*; SA, *Staphylococcus aureus*.
* Statistically significant.

Table 4. *Multivariate analysis of impact of epidemiological factors to carriage of* Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, *and* Staphylococcus aureus *in children undergoing adenoidectomy for recurrent upper respiratory tract infections*

Factors	Place of isolation	⩾1 or more pathogens		S. pneumoniae		H. influenzae		M. catarrhalis		S. aureus	
		OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Age (yr)	Nasopharynx	1·1 (0·6–2·0)	0·73	1·5 (0·9–2·4)	0·13	0·9 (0·5–1·5)	0·57	0·7 (0·4–1·2)	0·24	1·6 (0·9–2·9)	0·14
	Adenoid	2·8 (1·4–5·8)	0·004*	1·6 (1·0–2·7)	0·047*	0·7 (0·4–1·3)	0·27	1·0 (0·5–1·8)	0·89	1·5 (0·9–2·4)	0·16
Male sex	Nasopharynx	0·4 (0·2–0·7)	0·004*	0·7 (0·5–1·2)	0·73	0·9 (0·5–1·4)	0·59	0·5 (0·3–0·9)	0·014*	0·9 (0·5–1·5)	0·66
	Adenoid	0·6 (0·2–2·3)	0·46	0·8 (0·5–1·3)	0·32	1·0 (0·6–1·6)	0·94	0·5 (0·3–0·9)	0·015*	1·1 (0·7–1·7)	0·78
Sibling possessing	Nasopharynx	0·6 (0·3–1·4)	0·27	0·9 (0·5–1·7)	0·84	0·7 (0·4–1·2)	0·17	1·0 (0·5–1·9)	0·99	1·2 (0·6–2·5)	0·55
	Adenoid	1·7 (0·5–5·8)	0·39	0·9 (0·5–1·6)	0·67	1·0 (0·5–1·9)	0·98	1·4 (0·6–3·2)	0·48	0·8 (0·4–1·3)	0·35
Passive smoking	Nasopharynx	1·7 (0·9–3·2)	0·1	1·5 (0·9–2·3)	0·65	1·8 (1·1–2·9)	0·029*	1·3 (0·8–2·2)	0·33	0·9 (0·5–1·6)	0·79
	Adenoid	1·5 (0·3–8·3)	0·64	1·1 (0·7–1·7)	0·82	0·9 (0·5–1·5)	0·68	1·8 (1·0–3·3)	0·057	0·9 (0·6–1·5)	0·83
Rural residence	Nasopharynx	0·5 (0·3–0·9)	0·024*	0·6 (0·4–0·9)	0. 015*	0·8 (0·5–1·3)	0·30	1·3 (0·8–2·2)	0·24	0·6 (0·3–1·0)	0·044*
	Adenoid	0·7 (0·2–2·4)	0·53	0·7 (0·4–1·1)	0·085	0·9 (0·5–1·4)	0·56	0·6 (0·3–1·1)	0·08	0·9 (0·6–1·4)	0·64
DC attendance	Nasopharynx	1·3 (0·6–2·5)	0·5	1·2 (0·7–2·2)	0·55	0·5 (0·2–0·8)	0·010*	0·7 (0·4–1·3)	0·27	1·1 (0·5–2·2)	0·78
	Adenoid	1·0 (0·3–4·0)	0·98	0·8 (0·4–1·4)	0·45	0·6 (0·3–1·3)	0·23	0·7 (0·3–1·4)	0·30	1·5 (0·8–3·0)	0·23
Antibiotics taken for last episode											
Amoxicillin/co-amoxiclav		Referred		Referred		Referred		Referred		Referred	
Cephalosporins	Nasopharynx	1·1 (0·5–2·5)	0·86	1·0 (0·5–1·9)	0·95	0·8 (0·3–1·7)	0·49	0·9 (0·4–2·0)	0·83	0·9 (0·4–2·0)	0·80
	Adenoid	0·1 (0·03–0·8)	0·027*	1·2 (0·6–2·3)	0·63	0·3 (0·2–0·7)	0·003*	0·9 (0·3–2·4)	0·82	0·7 (0·4–1·6)	0·45
Macrolides	Nasopharynx	0·9 (0·4–2·2)	0·89	0·8 (0·4–1·6)	0·55	1·0 (0·4–2·1)	0·91	0·8 (0·3–1·8)	0·57	1·4 (0·6–3·0)	0·42
	Adenoid	1·5 (0·1–19·3)	0·76	0·6 (0·3–1·3)	0·20	1·6 (0·7–3·4)	0·26	0·8 (0·3–2·2)	0·66	1·0 (0·5–2·2)	0·91

OR, Odds ratio; CI, confidence interval; DC, daycare centre.
* Statistically significant.

of one or more pathogens in the nasopharynx. Correspondingly, adenoid colonization was found to be significantly associated with age ($P = 0.004$) and decreased by cephalosporin therapy for the most recent infection episode ($P = 0.027$). Rural residence significantly decreased the incidence of nasopharyngeal colonization with *S. pneumoniae* ($P = 0.015$) while this species in adenoid tissue was independently associated with age ($P = 0.047$). Passive smoking ($P = 0.029$) and not attending a daycare centre ($P = 0.01$) were the factors predisposing to nasopharyngeal carriage of *H. influenzae*. In the case of *H. influenzae* in adenoid samples, cephalosporins taken for the last infection episode significantly reduced its incidence. Female gender appeared to predispose to *M. catarrhalis* colonization in both the nasopharynx and adenoid tissue. The presence of *S. aureus* in the nasopharynx of subjects resident in rural areas was noted ($P = 0.044$), but no associated factor was evident for this organism in adenoid samples.

DISCUSSION

The most common cause of visits of paediatric patients in primary healthcare practice is pharyngotonsillitis or adenotonsillitis most often caused by viruses and group A streptococci. Treatment failure or recurrent infection is a common problem, but the presence of other agents is rarely considered. The adenoids of healthy children contain polymicrobial aerobic and anaerobic flora, including the main respiratory bacterial pathogens (i.e. *S. pneumoniae*, *S. aureus*, *H. influenzae*, and *M. catarrhalis*). Adenoids play a key role in antigen sampling and immune response induction directed against respiratory pathogens. Some organisms, however (e.g. *H. influenzae* [11], *M. catarrhalis* [12], and *S. aureus* [13]), deploy mechanisms to use pharyngeal lymphoid tissues as a niche to persist in the host. These pathogens are also highly associated with colonization of the adenoids in patients with recurrent otitis media, adenotonsillitis, and obstructive adenoid hyperplasia [6]. Our study confirms a high frequency of colonization by these organisms in patients with recurrent inflammations [9].

As previously noted [14] we also found a high prevalence of *H. influenzae* in the adenoids. This species is commonly recovered from recurrent tonsillitis in children [15], in removed tonsils due to recurrent and hyperplastic tonsillitis [16, 17] as well as in the nasopharynx in patients with pharyngotonsillitis [18]. *H. influenzae* is not clearly implicated as a cause of pharyngotonsillitis, but some studies have reported it in acute pharyngotonsillitis, sore throat, treatment failure or recurrent infections [10, 18]. Its isolation from adenoid specimens is also reported to be significantly higher from children with otitis media with effusion [19] and chronic otitis media [20] than from children without these conditions. We found *H. influenzae* more frequently from the adenoids than from the nasopharynx, suggesting involvement with inflamed adenoids. Moreover, none of the patients showed nasopharyngeal colonization with *H. influenzae* alone and so its isolation would be highly predictive of the aetiology of recurrent URTI. On the other hand, the absence of *H. influenzae* in the nasopharynx in these children does not exclude it as a cause of adenoid infections (low NPV).

The second most frequent pathogen was *S. pneumoniae* with a higher detection rate in adenoid samples than the nasopharynx (62.1% *vs.* 47.6%). This suggests that the adenoids in children with recurrent URTIs, may be the main reservoir of *S. pneumoniae* where it gives rise to chronic infection, swelling and inflammation. Asymptomatic nasopharyngeal carriage of pneumococci is widely prevalent in young children and is an important factor for transmission within a community and development of pneumococcal disease; as many as 60% of healthy preschool children have been shown to carry pneumococci in the nasopharynx [5]. Increased carriage rates in children have been associated with age during respiratory tract infections in the absence of otitis media [21] but other studies have found no difference in the overall *S. pneumoniae* carriage rate between healthy and sick children in different age groups [22] nor have been able to confirm the species as an important causative agent of respiratory tract infection and sore throat. From our data, the presence of *S. pneumoniae* in the nasopharynx had high specificity and PPV as a cause of recurrent URTI.

M. catarrhalis is a human-restricted commensal organism that over the last two decades has developed into an emerging respiratory tract pathogen [8]. The species may be overlooked in culture because of its phenotypic similarity to commensal *Neisseria*, which are part of the normal flora of the upper respiratory tract. Currently available data on age-specific colonization rates indicate that infants are exposed to *M. catarrhalis* very early in life [23], whereas carriage in older children is lower, with reported rates of 20–54% [24, 25]. *M. catarrhalis* has been found in tonsils or in the adenoids in children with recurrent

adenotonsillitis [14], and with recurrent otitis media [2]. We found a frequency of 28% for nasopharyngeal colonization in preschool children which is similar to the report of Zemlickova *et al.* [24] for the same age group (22·1%). No increased prevalence of *M. catarrhalis* in patients with respiratory tract infection and sore throat compared to healthy individuals has been reported [10] which possibly suggests a benign role for the organism. In our study, *M. catarrhalis* was the one pathogen which was more frequently isolated from the nasopharynx than adenoid samples, which could support this statement. However, Heiniger *et al.* [12] demonstrated that adenoids and tonsils from patients without acute respiratory tract disease harbour a subepithelial and intracellular reservoir of *M. catarrhalis* which may explain the relatively poor sensitivity of adenoid surface culture. The frequency of *M. catarrhalis* in adenoid tissue obtained in our study (18%) was similar to that observed in adenoids in children with otitis media with effusion (11–17·1%) [9, 26].

The *S. aureus* recovery rate in our patient cohort (33%) is in accordance with other reports of nasopharyngeal carriage of 10–35% of children in whom it can on occasion give rise to respiratory infection [1, 24]. *S. aureus* is one of the predominant species recovered from adenoid tissue in children with recurrent otitis media (10–46·7%), recurrent adenotonsillitis (10–40%) [6, 14, 27], and rhinosinusitis (15·6%) [28].

Bacterial pathogens are generally studied individually, although in their natural environment they often co-exist or compete with multiple microbial species. In this study, for the first time, relationships were compared between the four main pathogens during multiple colonization in the nasopharynx and adenoids. Multiple pathogens were found more commonly than single species in adenoids ($P < 0.0001$, OR 1·9, 95% CI 1·4–2·6) which is in accordance with most reports [9, 27]. However, for nasopharyngeal colonization, there was a similar number of samples yielding single or multiple pathogens. No associations in co-colonization between species was evident in the nasopharynx whereas in adenoids several positive associations were found, most notably between *S. pneumoniae* and *H. influenzae*, and others (Table 3). Some studies have reported an inverse correlation between nasopharyngeal carriage of *S. pneumoniae* and *S. aureus* [29, 30] which we also observed in our study but without statistical significance. Positive associations have been reported between pair-wise combinations of *S. pneumoniae* and *H. influenzae* and

between *S. pneumoniae* and *M. catarrhalis* but not for *S. pneumoniae* and *S. aureus*, or *H. influenzae* and *S. aureus* [7]. This is supported by our data, although we found statistically significant positive associations between *S. aureus* and *H. influenzae* or *S. pneumonia*, but not the reverse. Xu *et al.* [31] found a synergistic association between *S. pneumoniae* and *M. catarrhalis* only in healthy children, in contrast to children with acute otitis media where competitive associations between *H. influenzae* and *S. pneumoniae* or *M. catarrhalis* were evident. A fundamental difference between our data and previous reports is that these studies were confined to nasopharyngeal cultures. In the adenoids, the target pathogens are localized not only on the mucosal surface but also within crypts in the tissue in aggregates of cells surrounded by a carbohydrate matrix which is characteristic of bacterial biofilms [20]. Interactions between species and their virulence factors may be reduced and so it is difficult to determine the relationships, especially competitive interactions under these conditions.

Although it has been suggested that nasopharyngeal culture has significant utility for the choice of antibiotics in children with chronic adenoiditis [32], there are no data concerning the value of nasopharyngeal cultures in identification of the potential pathogens responsible for inflammation in adenoids and their predictive value for the aetiology of URTIs. In our study, these data were satisfactory only for *H. influenzae* and *S. pneumoniae* with high specificity but low sensitivity. However, our findings suggest that *M. catarrhalis* could be excluded as a cause of adenoiditis when not cultured from a nasopharyngeal swab due to a high NPV with good to high levels of sensitivity and specificity.

Passive smoking is associated with upper and lower tract infections, and also increases colonization by pathogens in children but the impact of tobacco exposure and carriage of pathogens is a matter of debate. Principi *et al.* [4] concluded that tobacco smoke exposure of healthy children aged <5 years did not influence pathogen carriage in the upper respiratory tract but a more recent study did show increased *S. pneumoniae* carriage rates in children exposed to tobacco smoke [33]. By contrast, Bakhshaee *et al.* [34] found a significant difference in carriage rates between children living in smoking families, compared to those in non-smoking families for *M. catarrhalis*, but not for *S. pneumoniae* and *H. influenzae*. In our cohort, passive smoking was a risk factor for increased nasopharyngeal colonization of *H. influenzae* and this

is supported by Kosikowska *et al.* [35] who also found increased colonization of this pathogen in healthy children aged 3–5 years, heavily exposed to tobacco smoke, as well as vulnerability to recurrent respiratory infections.

Our data suggest that place of residence of the child is a significant factor for microbial colonization, especially *S. pneumoniae* and *S. aureus* in the nasopharynx. Living in a rural area has been reported to be associated with less nasopharyngeal carriage of *S. pneumoniae* and *H. influenzae*, in children aged <5 years [4] but this was not supported by Boken *et al.* [36] who found no difference in the carriage rate of *S. pneumoniae* between rural- and urban-dwelling children. Surprisingly, in our study, children aged 4 and 5 years appear to have a higher prevalence of *S. pneumoniae* in the adenoids compared to younger subjects. Available data concerning age and other risk factors of pharyngeal colonization alone in children suggests that after peaking at 2–3 years of life, colonization with respiratory pathogens decreases with age [24, 37]; the <4 years age group may be more frequently colonized by *M. catarrhalis* while *S. aureus* carriage rate is more prevalent in older children [38]. Zemlickova *et al.* [24] demonstrated in healthy children aged 3–6 years that younger children (aged 3 years) were more frequently colonized by *S. pneumoniae* but this declined and was accompanied by a simultaneous increase in *S. aureus* at age 6 years. In our study, the peak incidence of *S. pneumoniae* and *S. aureus* was recorded in 5-year-olds, when the highest prevalence of *H. influenzae* and *M. catarrhalis* was at age 3 years. This difference may arise from the individuality of our studied groups, i.e. children undergoing adenoidectomy for recurrent URTIs and the specificity of the studied samples.

A significant relationship between cephalosporin treatment for the last infection and adenoid colonization by the target pathogens was evident particularly for *H. influenzae*. Antibiotic therapy has been cited as a significant contributor to the decrease in colonization rate of *S. pneumoniae* alone or in combination with *M. catarrhalis* in children with UTRIs, but not of *H. influenzae* [30, 39]. However, antimicrobial therapy has also been implicated in a decrease of each of these species in another study [40].

In conclusion, we have shown that adenoids can act as a reservoir of respiratory bacterial pathogens, especially *H. influenzae* and *S. pneumoniae*, in children undergoing adenoidectomy for recurrent URTIs.

However, the colonizing flora of adenoids and nasopharynx in the same patient are not always comparable. Nevertheless, the recovery of *H. influenzae* and *S. pneumoniae* in the nasopharynx could be predictive for the aetiology of URTIs. Prospective clinical trials are needed to determine the impact of adenoidectomy on composition of nasopharyngeal bacterial flora after surgery and its preventive role in possible future URTIs (i.e. acute otitis media, pharyngotonsillitis, rhinosinusitis) in young children.

DECLARATION OF INTEREST

None.

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