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LARYNGOLOGY

Adenoid bacterial colonization in a paediatric population

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Abstract Adenoids play a key role in both respiratory and ear infection in children. It has also been shown that adenoidectomy improves these symptoms in this population. The main goal of the present study was to evaluate adenoid bacterial colonization and document a possible relation with infectious respiratory disease. A prospective observational study was designed to evaluate the proposed hypothesis in a paediatric population submitted to adenoidectomy by either infectious or non-infectious indications and compare these two cohorts. A total of 62 patients with ages ranging from 1 to 12 years old were enrolled in the study. Adenoid surface, adenoid core and middle meatus microbiota were compared. A close association between adenoid colonization and nasal infection was found, supporting that adenoids may function as bacterial reservoir for upper airway infection. The obtained results also contribute to explain the success of adenoidectomy in patients with infectious indications.

Keywords Adenoidectomy · Nasal and adenoidal microbiota · Upper airways infection · Children

Introduction

The adenoid tonsil plays a key role in respiratory disease in children, be it infectious or non-infectious [1]. In children, adenoid hypertrophy is associated with acute rhinosinusitis in 32.9% of the cases. On the other hand, there is a high coexistence of adenoid infection and nasal sinusitis especially in children with ages between 2- and 5-year-olds [1]. Also it has been shown that adenoidectomy improves symptoms of chronic sinusitis in children [1]. A potential explanation for these findings is the presence of biofilms on adenoid surface [2]. There is growing evidence that adenoid core bacteria share identity with the biofilm forming bacteria on the adenoid surface and in the middle *meati*, and may, therefore, be responsible for respiratory disease [3–5]. Knowing this, the present study aims to contribute to the body of knowledge, comparing bacterial population in both adenoid core and surface, and in the middle *meati* of children having adenoid surgery. Furthermore, the evaluation of a possible relationship between adenoid colonization and infectious respiratory disease, comparing two populations either with infectious and non-infectious surgical indication is also a goal of the study.

Materials and methods

Study design and ethics

A prospective observational study was designed to evaluate the importance of nasopharyngeal and adenoid

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colonization on ear and upper respiratory infections in a paediatric population of a tertiary Hospital in Greater Lisbon, Hospital de Beatriz Angelo (HBA).

The study was approved by HBA Medical Ethics Board in accordance with the World Medical Association Declaration of Helsinki. An informed consent was obtained from the children tutors to allow the collection of 3 different samples (nasal swab, superficial adenoid swab and adenoid core biopsy) for microbiota study. Data related to clinical and children status were also collected.

Opportunity sampling was used for a 1 year period, enrolling 62 children undergoing adenoid surgery, divided in two cohorts: children with an infectious diagnosis like recurrent acute upper airway infection (otitis media, adenoiditis, tonsillitis and sinusitis), chronic rhinosinusitis, adenoiditis or chronic purulent otitis media (Group 1); and children with non-infectious diagnosis (obstructive sleep apnoea, chronic otitis media with effusion—Group 2).

Nasal swabs were collected during surgery from the middle *meati*, while protecting from the nasal fossae mucosa using a sterile nasal speculum. Adenoid surface swab was collected after retracting the palatal velum providing frank and unobstructed access to the adenoid surface. Adenoid core sample was collected through the same path, after the first curettage provided access to the deeper adenoid layers, taking special care not to collect surface fimbriae on the raw surface.

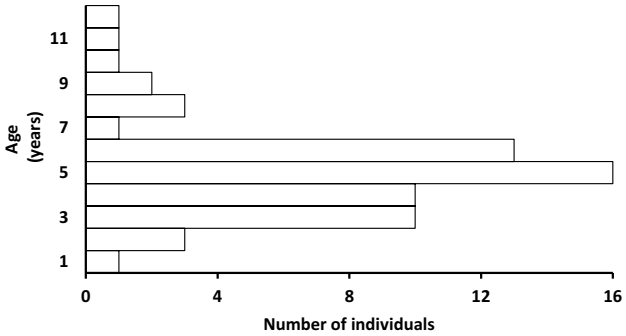

Fig. 1 Age distribution. The age distribution of the 62 individuals enrolled for the study is shown

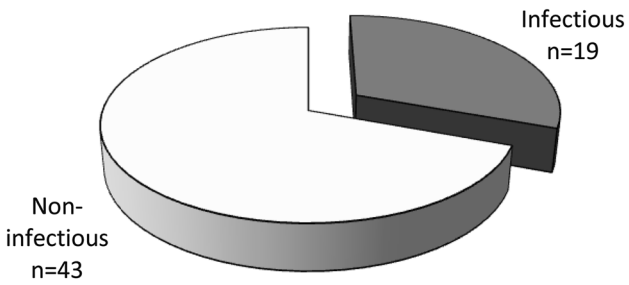

Fig. 2 Adenoidectomy indications. The population was divided in two cohorts according to the nature of the indication for adenoidectomy: a cohort of 19 infectious patients and another of 43 non-infectious

Bacterial isolation and identification

The nasal and adenoidal swabs were inoculated into brain heart infusion (BHI) and the adenoidal biopsies into phosphate buffer saline (PBS) and processed within the next 8h. The swabs and biopsies were homogenised and plated on Columbia agar with 5% sheep blood (COS) for isolation of non-fastidious bacteria; Chocolate PolyViteX agar (PVX) for isolation of fastidious bacteria; Chocolate PolyViteX VCAT3 agar (VCA3) for isolation of *N. meningitidis*; Mac Conkey agar (MC) for isolation of gram-negative bacilli; Columbia CNA agar with 5% sheep blood (CNA) for isolation of staphylococci and streptococci and Schaedler agar with 5% sheep blood (SCS) for isolation of anaerobic bacteria. The PVX and VCA3 were incubated at 37 °C in a 5% CO_2 atmosphere; SCS was incubated at 37 °C in an anaerobic atmosphere and all the others were incubated at 37 °C. In all the cases the cultures were incubated until 48 h being monitored after 18 h. The bacteria were further cultivated to obtain pure cultures and were identified using VITEK2 (BioMérieux). All the culture media were purchased from BioMérieux.

Statistical analysis

Unadjusted association between surgery indication and identity of the 4 major genera on nasal surface, adenoid surface and core microbiota were evaluated by Chi-square test. A p value inferior to 0.05 was considered statistically significant.

Results

Population data: 62 patients were enrolled in the study (28 females and 34 males) with ages ranging between 1 and 12 years old (Fig. 1). The majority of the population (79.0%) had between 3 and 6 years old. The mode corresponds to the age of 5 years old representing 25.8% of the individuals.

The population was divided in two cohorts according to surgery indication: Group 1 ($n=19$) infectious indication and Group 2 ($n=43$) non-infectious indication as shown in Fig. 2. In Group 1 the major surgery indications were recurrent acute upper airway infection (otitis media, sinusitis, adenoiditis, alone or associated with other diagnosis), chronic rhinosinusitis or chronic purulent otitis media;

non-infectious diagnosis were obstructive sleep apnoea or chronic otitis media with effusion (Group 2). All the participants, with the exception of one, were not subjected to antibiotic therapy within the previous month to adenoidectomy.

A normal distribution was observed for the age of individuals enrolled for both groups with equal values of median, mode and average. Nevertheless, the age modes calculated were 6 and 5 years old for Group 1 and 2, respectively.

Microbiota: From 62 patients were taken 186 samples, including adenoid core, adenoid surface and nasal swab from each patient being identified 33 bacterial genera, including potential pathogenic bacteria such as *Streptococcus pneumoniae, Haemophilus influenzae*, beta-hemolytic streptococci, and *Staphylococcus aureus*. Commensal species, relatively stable flora members, enterococci, non-hemolytic streptococci, *Neisseria* spp., coagulase-negative staphylococci and *Moraxella* spp. were also found. On aerobic culture, the genera *Haemophilus, Neisseria, Staphylococcus* and *Streptococcus* were frequently isolated in both groups, and Group 2 population (non-infectious indications $n=43$) exhibits a higher bacterial diversity in all samples than Group 1 (infectious indication $n=19$). As shown in Table 1 *Streptococcus* spp. (40.35%) and *Staphylococcus* spp. (19.38%) were predominant for Group 1 and 2, respectively. *Haemophilus genus* was more frequent in Group 1 (19.3%) than in Group 2 (17.83%) being *H. influenzae* by far the most important species. *N. meningitis* was isolated only from one patient with obstructive sleep apnoea syndrome (Group 2). Commensal *Neisseria* species were found in 19.29% and 12.61% of samples from Group 1 and 2, respectively.

Among 42 children (10 in Group 1 and 32 in Group 2), were simultaneously identified the same bacterial species on the adenoid core and at least one other sample (adenoid surface or nasal surface). The most representative species—*H. influenzae, S. aureus, S. pneumoniae* and *S. pyogenes*—were identified in 18 children simultaneously on the adenoid (core and surface) and on nasal samples; in contrast, from 14 children these species were isolated only on the adenoid core and surface. Group 2 exhibited a greater bacterial diversity in all samples than group 1 population (Fig. 3). From Group 1 nasal swabs were isolated 9 different bacterial species: *S. aureus* (50%) and *H. influenzae* (30%) the most frequent ones, followed by four species each with 10% (Fig. 3a). In Group 2, *S. aureus* (37.5%) and *H. influenzae* (28.1%) were also the most frequent, followed by *Corynebacterium pseudodiphtheriticum, S. pneumoniae* and other *Streptococcus* spp. each with 15.6% (Fig. 3b). In addition, seven other bacterial species were identified in lower percentages (Fig. 3b). The same trend of greater bacterial diversity in Group 2 than in Group 1 was observed

Table 1 Major microbiota genera

Bacteria	Group 1 (%)	Group 2 (%)
Haemophilus influenzae	15.79	13.18
Haemophilus parainfluenza	–	2.33
Haemophilus influenzae/parainfluenza	–	0.78
Haemophilus spp.	3.51	1.55
	19.30	17.83
Neisseria meningitidis	–	0.78
Neisseria cinerea	1.75	3.10
Neisseria lactamica	1.75	0.78
Neisseria sicca	5.26	0.78
Neisseria spp.	10.53	7.75
	19.29	13.39
Staphylococcus aureus	15.79	14.73
Staphylococcus cohnii	–	0.78
Staphylococcus epidermidis	1.75	1.55
Staphylococcus hominis	–	2.33
	17.54	19.38
Streptococcus alactolyticus	–	0.29
Streptococcus constellatus	–	0.29
Streptococcus cristatus/ sanguinis	3.51	–
Streptococcus pyogenes	1.75	2.57
Streptococcus Grupo C	3.51	1.14
Streptococcus hominis	–	0.29
Streptococcus intermedius	1.75	–
Streptococcus mitis/oralis	8.77	3.15
Streptococcus parasanguinis	–	1.14
Streptococcus plurianimalium	5.26	0.29
Streptococcus pneumoniae	5.26	3.72
Streptococcus pseudoporcinus	–	0.86
Streptococcus salivarius	7.02	2.00
Streptococcus sanguinius	3.51	0.29
	40.35	16.01

both on adenoid surface (Fig. 3c, d) and core samples (Fig. 3e, f). A total of 8 and 14 different bacterial species were isolated from adenoid core and surface of Group1 and 2, respectively. *H. influenzae, S. aureus* and other potential pathogens such as *S. pneumoniae, S. pyogenes* and *N. meningitidis* were identified in all the sub-sites on more than one sample.

A possible association between adenoid surface and nasal colonization and adenoid core invasion by bacteria was evaluated through chi-square test. In Group 1, a significant statistical associations was found for *Haemophilus, Neisseria, Staphylococcus* and *Streptococcus* genera on nasal and adenoid surface ($p<0.00001$), on adenoid surface and core ($p<0.00001$) and on the nasal surface and adenoid core ($p=0.000126$). On the other hand, in Group 2 significant associations between nasal surface and both adenoid surface ($p<0.00001$) and core ($p=0.000091$) were

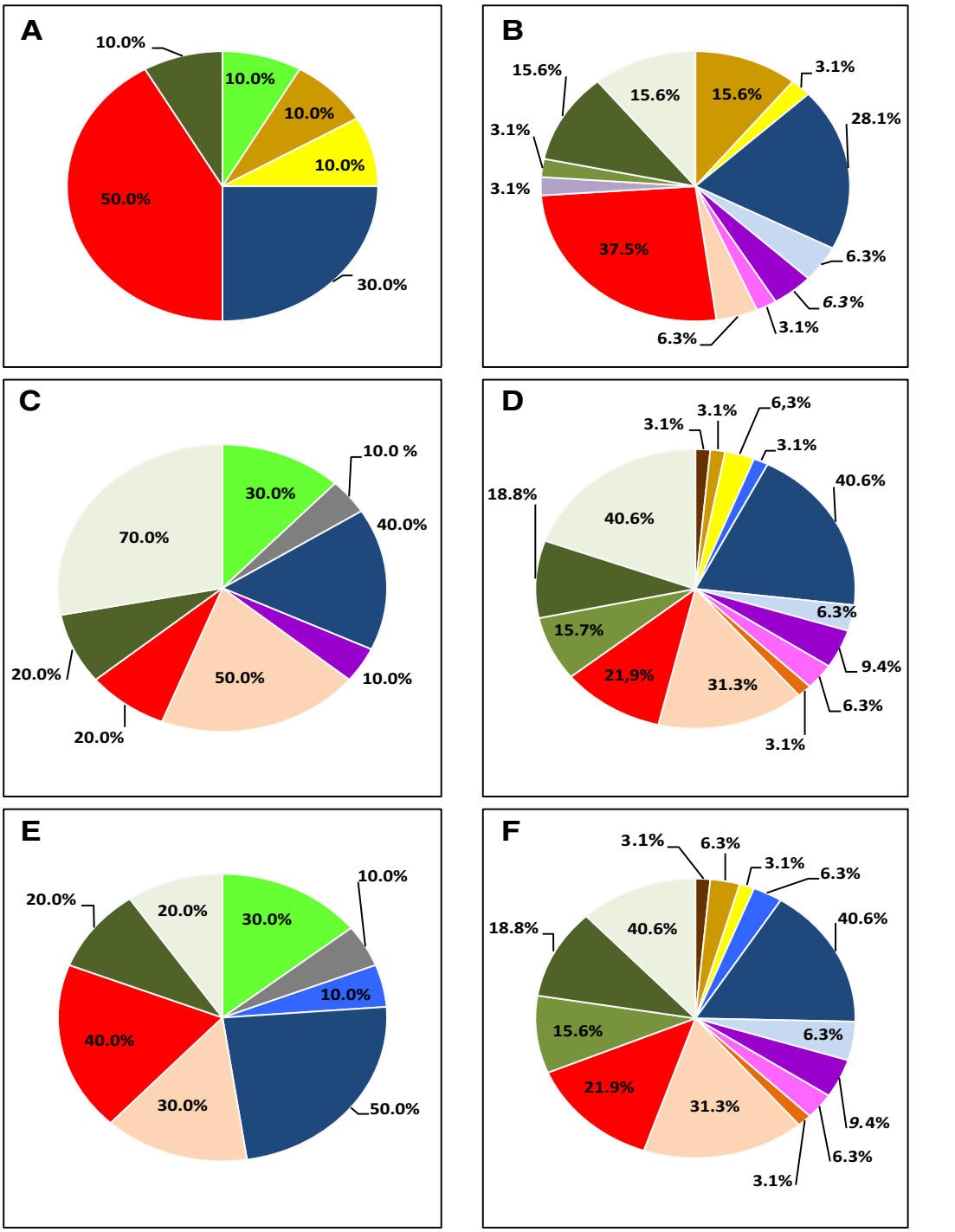

Fig. 3 Bacterial identity. The nasal, adenoid surface and core microbiota are shown in **a, c** and **e** for Group 1, respectively. In **b, d, f** is shown the microbiota of nasal, adenoid surface and core of Group 2

found. No significant association was found between adenoid surface and core flora ($p = 0.656$), at least for the main genera of bacteria.

Discussion

The identity between adenoid tonsil and upper airway bacterial colonization is known for some time [1], mostly from indirect evidence or from bacterial studies after the adenoid tissue was removed from the patient [3–5]. In the present work this issue was overcame by collecting core samples directly in situ, after removing a fragment from the adenoid surface. Unlike the palatine tonsils, adenoid tonsils in most cases are not as thick and have an irregular surface, making it difficult to clearly separate core from surface sampling. For this reason all samples that were not thick enough to provide a safe distinction between the two sources were excluded. This procedure allowed avoiding bias in the results.

Surface colonization has recently gained importance after being described the possible involvement of surface biofilms on upper airway infections [2, 6–9]. Because of this, core colonization has lost relevance in the eyes of researchers, in contrast to what has been happening in palatine tonsils, where core bacteria are a major concern [10]. This knowledge is important, because when diagnosing tonsil disease, it is not possible to rely surely on surface swabs, and the same may apply to adenoid tonsil disease. Knowing that these interactions are of paramount importance in explaining the causality of adenoid and upper airway infection, it could be presumed that adenoids function as a reservoir for re-infection as previously postulated [3, 11]. The bacterial genera found in this study are similar to those previously described, namely *Haemophilus, Staphylococcus and Streptococcus* [3, 12]. *Streptococcus* was the most frequent bacteria found, therefore, associated with infectious diagnosis [1]. Finding *Staphylococcus* as the most frequent bacteria in non-infectious cases was also not a surprise, since it is ubiquitous and known for being a nasopharyngeal commensal [13].

What has emerged as unusual was the identification of *N. meningitidis* not reported as a relevant infectious agent neither in pharynx nor in nasal cavities, but is certainly related to other serious infections, namely meningitis [14]. For this reason, our finding deserves prompt attention. Although not usual, it has been previously reported [15] and is, in our mind, not an innocent bystander in these patients.

Other interesting result was finding no statistically significant association between adenoid surface and adenoid core flora, at least for the main genera of bacteria, and this pairs with the majority of previous works on the palatine tonsils [5].

These results, therefore, come in line with previous reports describing that surface colonization in the adenoid tonsils may play a role in upper airway infections. This has medical and surgical therapeutical implications, for it contributes to explain the beneficial role of nasal irrigation [1, 16] and of adenoidectomy in children with these chronic infections [1].

In this study, the severity of the colonization was not quantified, since none of the participants were acutely inflamed, and therefore, bacteria were considered colonists and not infectious. To rule out adenoid inflammation the previously established criterion of mucous observed over the adenoid mucosa was used [15]. As such, the colonization was not quantified, and therefore, the bacteria were considered commensal instead of potentially pathogenic.

The most important limitation of this study was the number of participants and the asymmetry of the groups, with infectious indications (Group 1) being less common. Nevertheless, given the relative scarcity of literature on the subject, and also the innovative sampling method for the adenoid core, it is an important contribution for the state of the art.

Conclusion

A close association between adenoid surface colonization and nasal infection was found supporting the concept of adenoid tonsil as a bacterial reservoir for upper airway infection. This also contributes to explain the success of adenoidectomy in cases with infectious indications. Other interesting result was finding no significant association between adenoid surface and core flora, at least for the main genera of bacteria.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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Ethical approval The study was approved by Hospital de Beatriz Ângelo Medical Ethics Board in accordance with the World Medical Association Declaration of Helsinki.

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