PNEUMOCOCCI DETECTED IN SALIVA BY *lytA*-PCR WHEN PRESENT ARE AT VERY LOW DENSITY AND IN MOST CASES MAY BE NON-PNEUMOCOCCAL STREPTOCOCCI CONTAINING PNEUMOCOCCAL GENE HOMOLOGUES

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BACKGROUND AND AIMS

Predicting the impact of bacterial and viral vaccines on transmission of respiratory tract bacteria and thus population protection effects relies on non-invasive sampling techniques and rapid low cost laboratory analysis of large numbers of samples. Children (and adults) do not like nasal swabs. Obtaining saliva is easy, painless and can be done frequently, permitting detailed longitudinal studies. The presence of pneumococci (Sp), detected by PCR, in a high proportion of saliva samples from healthy children has previously been reported¹. Having obtained similar results, we analysed paired nasopharyngeal swab and saliva samples from children, in which both samples were *lytA* PCR positive using molecular serotyping by microarray.

METHODS

We obtained paired nasopharyngeal swab (NPS) and saliva samples from 95 healthy pre-school children, aged 6 months-6 years, attending daycare in Coimbra, Portugal in 2015. Samples were stored frozen in skim milk tryptone-glucose-glycerol (STGG) broth.

Bacterial culture: was done according to standard techniques for detection of *S. pneumoniae*. The residual growth from each plate was removed using a plastic loop into 1mL of glycerol broth and stored frozen at -80°C.

qPCR: DNA extractions from NPS and saliva culture-amplified samples were carried out using a QIASymphony Sp instrument and QIASymphony DSP Virus/Pathogen mini kit (937036, QIAGEN, Manchester, UK) according to the manufacturer's Virus Cellfree200 protocol. The PCR gene targeted was *lytA*. Samples were considered positives when cycle threshold (Ct) values were ≤35 and we used a previously published standard curve² to convert the Ct numbers at which a qPCR signal was detected into gene copies/mL.

Serotyping: we performed molecular serotyping by microarray on DNA extracts from the cultures, a method capable of detecting multiple serotypes and their relative abundance as previously described³.

RESULTS

49 paired NPS and saliva samples were *lytA* positive.

Among the 49 NPS samples, 45 showed evidence of *S. pneumoniae* by microarray, 3 showed only related non-*S. pneumoniae* streptococcus spp and one showed no evidence of *S. pneumoniae* or related species. In 28 there was evidence of a single *S. pneumoniae* serotype.

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RESULTS

By contrast, only 3/49 saliva samples had evidence of *S. pneumoniae* by microarray, in each case matching the *S. pneumoniae* serotype detected abundantly in the NPS sample from the same child but in all 3 at low abundance within a complex mix of other species (serotype 23A 95% vs 5%; serotype 35F 99% vs 4%; serotype 9N 100% vs 1%).

The mean number of streptococcal species suggested by the microarray analysis in the nasopharyngeal samples was 1.6 (median 1 (0-5)), whereas in the saliva samples it was 4.3 (median 5 (0-8)). Whereas evidence of species other than pneumococcus was found in a minority of NPS samples, it was the norm in saliva (table 1).

The relative predominance of non-pneumococcal species bearing capsular gene homologues detected by microarray in saliva as compared to NPS samples where pneumococci predominate, is shown in figure 1.

Table 1. Streptococcal species detected by microarray in nasopharyngeal and saliva samples.

Streptococcal species	In 49 <i>lytA</i> + nasopharyngeal samples	In 49 paired <i>lytA</i> + saliva samples
<i>S. pneumoniae</i> only	34	0
Non-pneumoniae Strep spp. only	3	44
<i>S. pneumoniae</i> + non-pneumoniae spp	11	3
No strep spp detected	1	2

Figure 1. Frequency of detection of pneumococcal vaccine (VT) and non-vaccine (NVT) serotypes, non-typeables (NT) and non-pneumococcal spp containing capsular gene homologues (non-Spn). NPS – nasopharyngeal swab samples.

CONCLUSIONS

- Nasopharyngeal and saliva samples from young children differ in their composition with regard to streptococcal species. The former frequently contain abundant pneumococci, usually without other streptococcal species, and mostly of a single or highly dominant serotype. The latter usually contain a mixture of several streptococcal species with pneumococci usually undetectable and when present, constituting a very small percentage of the streptococci in the sample.
- These results suggest that the common finding of positive *lytA* qPCR results in saliva samples in young children may, in reality, reflect the presence of genetically related streptococci from the oral flora, usually in complex mixtures, rather than pneumococci.
- Our findings do not definitively rule out the presence of small numbers of viable pneumococci whose presence is masked by the rich oral microbial flora. Nevertheless, they suggest that transmission of *S. pneumoniae* via saliva may be less important than via nasal secretions in this age group.
- Taken together with other recent literature demonstrating genetic similarity and overlap between pneumococci and other related streptococcal species⁴, this work emphasizes the practical and conceptual challenges faced when trying to define distinct boundaries between them.