Streptococcus pneumoniae (the pneumococcus, Spn) is leading bacterial cause of infections, such as pneumonia, meningitis and sepsis. Colonisation of the human nasopharynx with pneumococci is frequent, ranging from 40 to 95% in infants and 10-25% amongst the adults, but it is also the main reservoir of transmission and disease.

Using a human exposure model, we recently demonstrated that the hands can be vehicles for transmission of pneumococci, leading to acquisition of colonisation.

Now we use an experimental human pneumococcal challenge (EHPC) model to investigate the kinetics of pneumococcal shedding during a known initiation and duration colonisation episode and the role of pneumococcal density in shedding and transmission.

Methods

Experimental human pneumococcal challenge (EHPC)
Healthy volunteers were inoculated intranasally with S. pneumoniae, serotype 6B. Colonisation status and density were assessed by nasal wash lavages collected pre and at several time points post the inoculation (D2, D6, D16, D22, D27, D36) using classical microbiology.

Shedding samples
Post challenge, the volunteers were asked A) to cough twice on an agar plate (cough sample) and B) to rub the nose on their hand, following hand swabbing to assess shedding (nose-to-hand sample). Shedding samples were collected before the paired nasal wash sample at D2, D6, D16 and D22.

Pneumococcal shedding was quantified by classical microbiology. In nose-to-hand samples (collected in STGG), pneumococcal shedding was additionally quantified by 68 capsular polysaccharide specific qPCR.

Results

Pneumococcal (Spn) shedding rates per tested method

<table>
<thead>
<tr>
<th>Shedding method</th>
<th>Spn Sheddiers</th>
<th>% Shedding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nose-to-Hand</td>
<td>18/91</td>
<td>19.8%</td>
</tr>
<tr>
<td>Cough on plate</td>
<td>11/91</td>
<td>12.1%</td>
</tr>
<tr>
<td>Total</td>
<td>29/91</td>
<td>31.9%</td>
</tr>
</tbody>
</table>

In a total of 91 Spn colonised volunteers, 19.8% (18/91) expired bacteria from the nose to their hand and 12.1% (11/91) from the mouth while their were coughing at any time point from Day 2 to Day 22 after the pneumococcal challenge. Only 1 out of the 29 shedding expired bacteria from both nasal and oral route.

Increased cytokine responses in children (<5yrs) with detectable Spn colonisation in the nose than in the throat

In a separate study conducted in children (<5yrs), natural acquired pneumococcal colonisation was assessed by classical microbiology and IL6 qPCR. The average pneumococcal density was \(4 \times 10^5\) CFU/ml. Children with detectable pneumococci in nasosorption filter or both nasosorption and nasopharyngeal swab (NPS) had increased inflammatory responses compared to those kids that Spn colonisation was detectable only by NPS.

High pneumococcal colonisation density is associated with increased rates of bacterial shedding through the nose in adults

Volunteers colonised with pneumococcal (Spn) density \(<10^5\) CFU/ml of NW did not shed bacteria through the nose at any time point post the establishment of colonisation (non-shedders) (n= 133 nose-to-hand shedding samples).

Pneumococcal shedding from the nose was observed in only subjects who were colonised with Spn density above \(10^5\) CFU/ml (Spn shadders). In particularly, shedding rates were linearly increased at colonisation densities from \(10^3\) to \(10^5\) CFU/ml NW (n=44 nose-to-hand shedding samples)

100% shedding was achieved at any density between \(10^3\) and \(10^5\) CFU/ml NW.

Increased numbers of bacterial expiration through the nose in heavily colonised adults

In shedders, the nasopharyngeal pneumococcal density (quantified in NW samples at each time-point) was correlated with bacterial shedding ( amount of pneumococci quantified in the nose-to-hand sample by both classical microbiology and qPCR). Nasal pneumococcal density and pneumococcal shedding were positive correlated (\(R^2=0.59,\) **\(p=0.0026\) using Spearman correlation)

Conclusions and Perspectives

- In this study, we described for the first time that healthy human colonised adults can shed pneumococci. Therefore, they can also serve as a reservoir of transmission.
- We also demonstrated that density of colonisation is a major factor for bacterial shedding. Healthy adults colonised with Spn density \(>10^5\) CFU/ml NW at any time-point were more likely to shed bacteria through the nose. Colonised volunteers with a density from \(10^3\) to \(10^5\) CFU/ml NW - pneumococcal density commonly found in naturally colonised children- had the highest rates of pneumococcal shedding.
- We are currently examining primary viral infection and levels of inflammation in association with shedding rates.

Current vaccines, such as polysaccharide-conjugated pneumococcal vaccines (PCVs), have an important potential for reducing transmission and disease in heavily colonised groups by attenuating the density of vaccine included serotypes.