**Widespread use of Pneumococcal Conjugate Vaccines (PCV) has resulted in a reduction in nasopharyngeal colonisation and invasive pneumococcal disease caused by vaccine-type serotypes. In a double-blind, randomised controlled trial using the Experimental Human Pneumococcal Challenge (EHPC) model, PCV-13 (Prevenar-13) conferred 83% protection against colonisation acquisition of S. pneumoniae serotype 6B and a reduction in bacterial intensity in experimentally colonised volunteers as measured by classical culture.**

We used a qPCR approach to re-assess volunteer samples from our PCV study for experimental colonisation of 6B pneumococcus.

**DNA extraction**

DNA was extracted from nasal wash bacterial pellets using the Agowa mag Mini DNA isolation kit

**Multiplex qPCR**

We developed a novel multiplex qPCR based on methods previously published, using partial amplification of lytA and 6A/B cpsA genes. DNA from @HN418 serotype 6B, serially diluted 1:10 from 4.14x10^6 copies, was used as a standard curve. A sample was considered positive if at least one duplicate had a CT value less than 40.

**Colonisation acquisition rates by molecular methods**

We evaluated 193 samples from 90 volunteers and showed that PCV conferred 83% protection against experimental pneumococcal colonisation by classical culture and 29% protection by molecular methods.

**Colonisation densities by molecular methods**

Colonisation densities were significantly lower in volunteers vaccinated with PCV compared to the control arm by both classical culture (p=0.03) and molecular methods (p<0.0001). 91% of samples positive by lytA/cpsA qPCR but not by classical culture had densities <30 DNA copies/ml

**Correlation between densities calculated by classical culture and molecular methods**

1. We showed, by using molecular methods instead of classical culture, that protection conferred by PCV vaccination against experimental colonisation reduces from 83% to 29%. This may indicate that the main protective mechanism of this vaccine is mediated by reduction of colonisation density, leading to a decreased risk of disease to vaccinated individuals as well as transmission resulting in the observed herd effects in vaccinated populations.

2. Studies assessing the impact of pneumococcal vaccines should allow for density measurements in their design.

**CONCLUSIONS**

**References:**