**INTRODUCTION**

*Streptococcus pneumoniae* can be detected and typed by PCR-based methods utilizing the heterogeneity within the capsular operon. Subsequent multiplex typing assays have been expanded to cover more serotypes and to determine the quantity of DNA and therefore density of colonization. In Bulgaria PCV 10 was introduced in the year 2010 with coverage estimates of over 90% and presumably changed the pneumococcal serotype distribution.

Serotyping is currently needed for:

- Epidemiological surveys
- Effectiveness of the vaccine
- Tracking serotype replacement after vaccine introduction
- Emerging of non vaccine serotypes (NVT)

**METHODS**

For the detection of pneumococci, *cpsA* and *tetA* regions were amplified. PCR-based typing methods used in the survey included multiplex reactions:

- Real Time PCR – (21 primer pairs) 37 serotypes
- Conventional PCR – additional 20 primer pairs
- Commercial kit Operon S.PneumoStrip® - total 76 serotypes

A combination of the different types of PCR analysis were used to achieve the maximal number of typed samples:

- Real time PCR was used for fast screening for pneumococci in directly isolated DNA
- Directly isolated DNA was mainly analyzed with the qPCR because of the low quantity of DNA
- If the directly isolated DNA was in good quantity the sample was analyzed also with conventional PCR for the detection of more serotypes
- DNA from cultures was with good quantity and was analyzed with the conventional PCR

For the nontypeable strains and for subtyping of serogroups we used kit Operon S.PneumoStrip®

**RESULTS**

- Overall carriage from all samples (n=764) was 52% with culture positive 21%, this determines the molecular methods as at least two times more accurate than the culture-based methods
- 3.5% of samples with more than one serotype – 30 samples with two serotypes and 4 samples with three serotypes at the same time
- Below 10% of all positive samples were nontypeable for the primers and methods
- Vaccine type carriage detected was less than 5% of all positive results
- Prevalent carriage of two vaccine types was found - 4 and 23F, and 6C prevalence in non vaccine serotypes

**CONCLUSIONS**

In conclusion, PCR typing assays used are an accurate, rapid and reproducible for determining the serotype dynamics of pneumococcal populations.

- With the methods used we were able to type more than 90% of the serotypes/serogroups carried in the analyzed children.
- The methods are applicable for clinical settings and do not require culturing.
- Estimated vaccine effects were the absence of serotypes 6B and 18C and less than 1% distribution of serotype 19F.
- Although reduced, vaccine type colonization in children remains persistent eight years after the introduction of PCV10.