IN VITRO FITNESS ANALYSIS OF PNEUMOCOCCAL STRAINS BEFORE AND AFTER INTRODUCTION OF THE CONJUGATE VACCINE (PCV7) – WHAT ARE THE MOST ACCURATE PARAMETERS?

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Objective: The pneumococcal polysaccharide capsule is a major virulence factor and more than 90 pneumococcal (capsular) serotypes with different prevalence have been described. Based on multi-locus sequence typing, pneumococci can be distinguished in individual sequence types that can be grouped as cloncal complex. Serotypes do not equal sequence types, and isolates with different serotypes can be members of the same clonal complex.

Within several years, the children vaccination program with the heptavalent pneumococcal conjugate vaccine (PCV7) that included the most prevalent STs (4, 6B, 9V, 14, 18C, 19F, and 23F) has resulted almost in eradication of these 7 vaccine ST in many countries. Shortly after introduction of PCV7, an increase of the non-PCV7-ST 19A and the clonal complex 199 (CC199) was observed in many regions ('replacement'). Replacement can be explained by either selection advantage conferred by PCV7 or natural population shifts (i.e. the emergence of a successful clonal complex with superior biological fitness). Increase in ST 19A was e.g. described already in Denmark in 1973 – long before introduction of PCV7[1].

Methods: We analysed the *growth curves (XXX repetitions)* of the most prevalent STs before and after the introduction of the PCV7 in Germany in 2007. We analysed all invasive isolates sampled by the National Reference Center for Streptococci (NRZ, Aachen) during 2006-9: ST14 CC9 (n=37), ST6B CC176 (n=33), ST19A CC199 (n=27), 15 A CC199 (n=1), 15B CC199 (n=9), 15C CC199 (n=6) and ST3 CC180 (n=36).

Results: Statistical analysis indicated that the clonal complex discriminates the fitness stronger than affiliation to a certain serotype. Descriptive statistical analysis revealed maximal growth rates and maximal cell densities as most discriminative parameters between CC176 and CC180 isolates, whereas isolates of CC199 and CC9 showed highly overlapping values. Since CC176 and CC9 represent the dominating CCs before introduction of the vaccine and CC199 and CC180 are the major replacement CCs, these results suggest that fitness represented by growth rate is not the main driving force of the observed switch in serotypes.

Conclusion: In vitro fitness of pneumococcal isolates is rather determined by clonal complex than by serotype. Differences in vitro fitness between certain clonal complexes cannot sufficiently explain the observed serotype replacement after introduction of PCV7.

1 Harboe ZB, Benfield TL, Valentiner-Branth P, et al. Temporal trends in invasive pneumococcal disease and pneumococcal serotypes over 7 decades. *Clin Infect Dis*. 2010; **50**: 329-337.