Streptococcus pneumoniae: Serotype Diversity and Epidemiology

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Overview

- Capsule, serotype and antigenicity
- Global serotype distribution: pre- and post vaccine
  - Geographic region (Africa, Asia, Europe, Latin-American countries, N. America, & Oceania)
  - Host age group
- Serotype-specific disease & mortality
- The ecology of nasopharyngeal colonization
  - Serotype
  - Antibiotic susceptibility
  - Strain type (sequence type)

Pneumococcal serotype

- Based on structural and hence immunological differences in the polysaccharide capsule
- The capsule enables evasion of phagocytosis and immune responses
- Just over 90 serotypes exist but only ~ 1/3 cause infectious disease
- The capsule is synthesised by several genes located on a single capsulation locus ~20kbp long, bordered by the highly conserved genes, dexB and aliA
- Capsule synthesis can be switched ON or OFF – ‘phase variation’
- Capsule ‘switching’ can occur where the entire locus or individual genes are recombined to produce a different serotype
Capsule biosynthesis genes and repeat-unit polysaccharide structures

Capsule antigenicity
- Antigenicity thought to vary between serotypes...
- Some have a strong immunological response whilst others may not
- Thus, particular serotypes may colonise the nasopharynx for longer time periods
- Re-colonisation by the same serotype observed, indicating that a protective immunological response was not elicited on the first encounter, e.g., serotype 6
  - Leung, 2011
- The primary reservoir of *S. pneumoniae* is found in children < 5 yrs
- It is thought that with age, the child becomes exposed and develops resistance to the different serotypes;
  - In teenage years, both carriage and disease decrease significantly
- Majority of serotypes causing disease are also commonly carried

Global serotype distribution of IPD isolates among children < 5 years of age

The global level analyses of serotypes without adjustment—weighted by regional pneumococcal disease burden—Thus, serotype distribution is influenced by the regions or countries with the greatest amount of serotype data, which are not necessarily those with the greatest number of pneumococcal cases or deaths among children < 5 years of age
Global serotype distribution of IPD isolates among children < 5 years of age (2)

- Serotype 14 is the most commonly isolated serotype in children <5 years of age
- Serotypes 14, 6B, 1, 19F, 23F account for approximately 60% of all serotypes causing IPD in children <5 years old
- 11 serotypes account for >80% of all pneumococcal isolates reported
- Of 90 known pneumococcal serotypes, 15 not identified as a cause of IPD among children

Global serotype distribution by age group or by syndrome or body fluid

- Serotype 1 is more common in children 2-<5 yrs of age than the under 2’s years of age
- Serotype 14 is the most common from blood, CSF, pneumonia and meningitis
- Serotype 1 is more common in pneumonia than meningitis however, when age distribution is taken into account this could be reduced or eliminated

Regional serotype distribution

- Age is a potential confounder of serotype distributions across regions because:
  - Age and serotype distribution are associated (i.e., some serotypes are relatively more common among older children than among younger children)
  - Age and serotype distribution vary by region; Specifically a greater proportion of the isolates in data sets from Asia and Africa are from 2-<5 year olds than among the datasets from other regions
  - The proportion of serotyped isolates from children aged 2-<5 years of age is greatest in Asia and Africa, therefore summaries of serotype distributions among those “<5 year olds” will tend, relative to other regions, to emphasize serotypes causing disease in children aged 2-<5 years
Regional prevalence of serotypes

- Serotype 14 most common in children <5 in all regions except Asia where it and serotype 1 are co-ranked the most common
- In each region, the serotypes 1, 5, and 14 account for between 1/3 and 1/2 of disease in children <5 years old
- Serotype 1 is ranked in the top 4 serotypes in every region, except N. America and Oceania
- In all regions, among children <5 yo:
  - 3 to 5 serotypes account for >90% of isolates
  - 5 to 7 serotypes account for >85% of isolates
  - 7 to 11 serotypes account for >80% of isolates
- Africa and Asia share the same top 8 serotypes; The cumulative proportion of these 8 is somewhat higher in Africa than in Asia, with a range of approximately 70-80%

Regional serotype distribution by age

- In all regions, serotype 14 is the most common serotype in children <2yo
- In Asia and Africa, among children 2-<5yo, serotype 1 is the most common serotype;
  - In all other regions, serotype 14 is the most common serotype
- In all regions, serotype 1 is relatively more common or ranked higher in children 2-<5yo as compared to children <2yo
- N.B.: regional differences in the apparent proportion of disease due to serotype 1 may be partly explained by differences in the age distribution of the children <5 yo providing isolates;
  - While Africa and Asia appear to have a higher proportion of disease among children <5 yo due to serotype 1, they also have a higher proportion of isolates from children aged 2-<5 yo, and this may in part explain this difference

Serotype-specific disease & mortality

- Estimated by the proportion of pneumococcal disease caused by a given serotype (incidence of pneumococcal disease) x (case fatality rate)
  - This estimate includes cases and deaths from IPD and non-bacteraemic pneumonia
- Analyses of serotype specific incidence should be interpreted with caution because:
  - The assumption that the serotype distribution of IPD cases is similar to non-bacteraemic pneumonia cases
  - The case fatality rate does not vary by serotype
  - There are broad confidence intervals on the global disease burden case and death estimates

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**Serotype associated mortality**

<table>
<thead>
<tr>
<th>Serotype</th>
<th>No. (% of total)</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>46 (10.1)</td>
<td>6 (1.3)</td>
</tr>
<tr>
<td>3</td>
<td>24 (5.3)</td>
<td>12 (50)</td>
</tr>
<tr>
<td>4</td>
<td>49 (10.8)</td>
<td>11 (22.4)</td>
</tr>
<tr>
<td>6A</td>
<td>10 (2.2)</td>
<td>1 (10)</td>
</tr>
<tr>
<td>6B</td>
<td>12 (2.6)</td>
<td>5 (41.7)</td>
</tr>
<tr>
<td>7F</td>
<td>22 (4.8)</td>
<td>3 (13.6)</td>
</tr>
<tr>
<td>9</td>
<td>12 (2.6)</td>
<td>5 (41.7)</td>
</tr>
<tr>
<td>9N</td>
<td>13 (2.9)</td>
<td>3 (13.6)</td>
</tr>
<tr>
<td>9V</td>
<td>22 (4.8)</td>
<td>9 (39.1)</td>
</tr>
<tr>
<td>12F</td>
<td>18 (4.0)</td>
<td>5 (27.8)</td>
</tr>
<tr>
<td>14</td>
<td>27 (6.1)</td>
<td>9 (34.0)</td>
</tr>
<tr>
<td>19A</td>
<td>10 (2.2)</td>
<td>5 (50)</td>
</tr>
<tr>
<td>19F</td>
<td>12 (2.6)</td>
<td>4 (30.8)</td>
</tr>
<tr>
<td>20</td>
<td>10 (2.2)</td>
<td>5 (50)</td>
</tr>
<tr>
<td>23F</td>
<td>15 (3.3)</td>
<td>5 (31.2)</td>
</tr>
<tr>
<td>5, 10A, 10F, 11A, 12B, 15F, 19, 19F, 23F, 35F, 36, 42, 48 and 'rough'</td>
<td>74 (15.9)</td>
<td>27 (36.5)</td>
</tr>
<tr>
<td>Not typed</td>
<td>54 (11.2)</td>
<td>13 (24.1)</td>
</tr>
<tr>
<td>Total</td>
<td>464 (100)</td>
<td>123 (26.5)</td>
</tr>
</tbody>
</table>

Retrospective review of 464 cases of IPD in adults diagnosed between 1990 and 2001

Multivariate Cox proportional hazard analysis; Martens et al., BMC Infectious Diseases 2004, 4:21

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**Serotype-specific incidence by region**

- The estimated serotype-specific incidence rates vary substantially by region, e.g., serotype 1 varies in incidence from a low of <5 cases/100,000 in children <5 yo in N. America to >500/100,000 in Africa, a relative difference of over 100-fold
- In regions with high pneumococcal disease incidence (Africa, Asia, and LAC) the incidence of leading individual serotypes exceeds the overall incidence of pneumococcal disease in N. America
- In the regions with high pneumococcal disease incidence, the top 4 serotypes combined account for an estimated incidence of disease >1000 cases/100,000 children <5 yo

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**Serotype-specific mortality by region**

- The estimated serotype-specific mortality rates vary substantially by region, e.g., serotype 14, varies in incidence from a low of 1 case/100,000 children <5 yo in N. America to over 70 in Africa, a relative difference of over 70-fold
- In high incidence regions (Africa, Asia, and LAC) the mortality from leading individual serotypes exceeds the overall mortality of pneumococcal disease in N. America
- In Africa, the top 4 serotypes combined account for an estimated incidence of over 200 pneumococcal deaths per 100,000 children <5 yo
- The next highest mortality regions, Asia and LAC, the top 4 serotypes account for approximately 20-30 deaths per 100,000 children <5 years old

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**Epidemiology – pre-vaccine**

- In the absence of vaccines, the PCV7 serotypes (4, 6B, 9V, 14, 18C, 19F, and 23F) make up around 75% of invasive pneumococcal disease (IPD).
- In Malawi (sub-Saharan country) serotypes 1, 6A, and 6B constitute >40% of IPD in children\(^1\)


**Pneumococcal vaccines**

- The capsule is a target for vaccines.
- Protein-polysaccharide conjugate vaccines have been developed that elicit immunological memory in children as well as adults.
- 1999 - 7-valent (PCV7) vaccine
  (4, 6B, 9V, 14, 18C, 19F, and 23F)
- Currently - 13-valent (PCV13) vaccine
  (PCV7 + 1, 3, 5, 6A, 7F, and 19A) covering prevalent serotypes worldwide.

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**Epidemiology – post-vaccine**

- PCV7 vaccine - reduced PCV7 serotype disease incidence and led to replacement with non-vaccine serotypes (NVTs), e.g., serotype 6C.
- A study on paediatric isolates found high prevalence of 6C, with 44% of these isolated from blood\(^1\).
- Typing reveals that some serotype 6C strains were previously associated with vaccine serotypes 6B, 19A, and 23F, indicating capsule switching\(^1\).
- A post-PCV7 Spanish study found the NVTs 19A and 7F as the leading serotypes in IPD\(^1\).


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IPD and HIV+ve epidemiology
- IPD is one of the most common complications of HIV+ve individuals
- IPD incidence decreases by over 90% within 4 years of HAART antiviral therapy
- Remarkably, HAART therapy was superior to conjugate vaccination in reducing IPD in HIV+ve patients

How do serotypes differ between carriage and IPD?
- Some serotypes appear to be more invasive than others
- The prevalent serotypes identified in the nasopharynx of community-acquired pneumonia (CAP) children included 1, 5, 22F, 7F, 14, and 9V
- Odds ratio for colonization prevalence in CAP to healthy individuals indicated that serotypes 6A, 6B, 23A, and 35B, were lower suggesting that these serotypes have lower disease potentials
- In colonization, serotypes 1 and 5 increased with age, while serotypes 14 and 19F decreased, regardless of health status

Detailed nasopharyngeal ecology
- 11.5% of colonizations in children <6 yo had multiple serotypes, with up to 5 detected, i.e., 6B, 10A, 19A, 19F, and 231
- 4 of these also had co-colonisation of penicillin and/or co-trimoxazole susceptible and non-susceptible pneumococci
- An acapsulate strain with a deleted capsule locus observed1
- Strain typing revealed co-colonization with up to 6 strains with 2 strains having serotype 6B
- Genetically related strains in clonal complexes were circulating in this cohort
- This study revealed phenotypic and genetic evidence of microevolution and a greater diversity of pneumococcal strains colonising together than previously observed - increasing the potential for adaptation

References:

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Evolution during carriage

- It is during harmless carriage of the nasopharynx that evolution can occur.
- Disease causing pneumococci are evolutionary dead ends because they either kill their host, or the host with the aid of antibiotics kills it.
- The longer the duration of carriage the greater the opportunity to evolve.
- For example, serotypes that are successful colonisers such as 6B, are more likely to evolve antibiotic resistance, whereas serotype 1 that appear to be poor colonisers with a shorter duration of carriage have yet to evolve antibiotic resistance despite being prevalent in IPD.

Geographic distribution of quorum sensing pherotypes

- Carriage depends on adhesion to the mucosal epithelium and biofilm formation.
- The pherotype distribution was compared in strains from clinical and carriage strains from the UK and from Tanzania.
- CSP4 was 5-times more prevalent in Tanzanian strains compared with UK strains.
- CSP6.1 previously reported as a pneumococcal pherotype was shown by us to be a common pherotype in the new pathogen S. pseudopneumoniae.
- Unique clones were identified in Tanzanian strains suggesting that these geographic differences maybe due to limited inter-regional transmission.
- Tanzanian community live on a semi-closed sugar plantation.

Multiple pherotypes co-colonizing the nasopharynx

- Mediate competence:
  - Adhesion & biofilms on the mucosal surface
  - Fratricide (lysis of non competent cells with a different pherotype)
  - Up to 3 pherotypes colonise together
  - Potential for genetic exchange between different pherotypes within a colonisation
  - Unknown if the pherotypes are at a stable equilibrium (climax community), or whether they are replacing or re-emerging
  - Unknown if there is cross-talk between the different pherotypes leading to synergy or competition between the pherotypes living together
- Other oral streptococci also have similar QS systems so these may also be involved in fratricide and genetic exchange.
Conclusions

- A small number of serotypes cause disease and death in children under 5yo.
- Regional variations occur in disease and mortality rates, resource-poor regions have much higher rates, 70-200x greater.
- Introduction of serotype-based vaccines has been highly effective, but evidence of serotype replacement disease is emerging.
- Evolution occurs during carriage, and multiple strains co-colonising can increase the adaptive potential.
- Successful colonisation depends on adhesion and biofilm development triggered by quorum-sensing molecules, known as the competence stimulating peptide.
- Different variants of CSP have been identified and can co-colonise together.
- Much work still needs to be done to elucidate the influence of different phenotypes in harmless carriage and disease.