Molecular typing of pneumococci for the investigation of linked cases of invasive pneumococcal diseases

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Abstract:

In winter 2007/08, an outbreak of paediatric pneumonia caused by serotype 5 pneumococci was identified in a North East London suburb. Variable Number of Tandem Repeat analyses clustered these pneumococci from the other serotype 5 pneumococci in the UK, highlighting the importance of this discriminative typing method in supporting epidemiological investigations.
Streptococcus pneumoniae is the principal causative agent of community-acquired pneumonia. It is also an important cause of meningitis, septicaemia and otitis media.

Compared with outbreaks in closed settings such as hospitals or care homes where clusters of invasive pneumococcal disease (IPD) are easily identifiable, the situation is different in communities or geographically localised clusters of IPD. In this situation, cases are most often identified when the serotype involved is unusual for that time or place. In December 2007, three cases (cases 1-3) of paediatric pneumococcal pneumonia were reported from a hospital in Central London. Blood culture isolates from these cases referred to the Respiratory and Systemic Infection Laboratory (RSIL) were characterized as S. pneumoniae serotype 5. All of the patients lived in the same area of North East London, in a close-knit religious community. There were close social links between the families of these three cases.

Active surveillance of pneumonia cases admitted to this hospital over the succeeding weeks (January to March 2008) identified 5 more cases of clinical pneumonia in young children from this community. One of these was caused by serotype 3 pneumococcus and therefore not included in the cluster. Case 4 was regarded as probable as no sample was available for serotyping but had close social links with case 2. Blood cultures from case 5 and
case 6 yielded serotype 5 pneumococci. Case 7: a child with close social links to case 1 was admitted in March 2008 to the hospital with empyema; direct detection of specific antigen (11) from a pleural fluid sample identified the presence of serotype 5 capsular antigen. A further child (case 8) living 300 miles away from London in the North East of England was identified through the National Programme of Enhanced Pneumococcal Empyema Surveillance. The child was visited the week before becoming ill by her grandparents, who were members of the same religious community, and who normally reside in the NE London outbreak area. Direct detection of serotype 5 pneumococcal capsular antigen in a pleural fluid sample confirmed the diagnosis. In addition, two unrelated adults, living in the same London area but who were not from this religious community, developed septic shock and died. Urine samples from these two cases were also positive for serotype 5-specific pneumococcal antigen. No additional samples were available for further pneumococcal characterization. These two cases should be regarded as probable rather than confirmed.

Epidemiological investigations failed to establish direct contact between any of the cases, but there were close social links between several of the families (figure 1). This cluster of serotype 5 IPD was recognised as such because serotype 5 is rarely seen in the United Kingdom.
Serotype 5, like serotype 1 has been associated with outbreaks and clusters of invasive pneumococcal disease (1, 2) and these serotypes also tend to cause an increased proportion of complicated pneumonia cases (8).

In the NE London cluster, three of the children developed pneumococcal empyema and the two probable adult cases developed fatal septic shock.

Genetic characterization was performed to support the epidemiological investigations. Multi Locus Sequence Typing (MLST) and Multi Locus VNTR Analyses (MLVA) were performed on five pneumococcal cultures (case 1, 2, 3, 5, 6) and two DNA samples (case 7, 8) extracted from pleural fluid referred from the Meningococcal Reference Unit-HPA Manchester. In addition, all isolates (n= 28) from cases of serotype 5 IPD referred to RSIL during the period February 2003 – April 2008 from England and Wales were included in this study. MLST was performed as described previously (9) using the Bionumerics automated pipeline for sequence analyses (10). MLVA (6) was performed using a reduced number of VNTR loci (ms17, ms19, ms25, ms34, ms36, ms37 and ms 39). Selection of loci was based on best values of diversity indexes (4) of all possible combination of loci (5). PCR products were sized on 2% agarose gels and numbers of repeats were deduced from R6 sequence. Clustering analyses were performed using
MLST revealed that pneumococci of serotype 5 were genetically closely related. Thirty-two out of thirty-five shared the same allelic profile of sequence type (ST) 289. New STs were identified in 2 pneumococci: ST3468 and ST3844. ST3468 is a single locus variant (SLV) of ST289. ST3844 is not related to the other UK isolates but is closely related to ST3590 previously reported in MLST database (http://spneumoniae.mlst.net/) from South Korea in 2000. MLST analysis suggests the clonal expansion of isolates of serotype 5. Serotype 5 pneumococci are uncommonly detected in nasopharyngeal carriage samples even where they are a common cause of invasive disease (3), suggesting that colonisation of the nasopharynx is only transitory. Short periods of colonisation may reduce contact with other pneumococcal strains and therefore may limit genetic diversity within the serotype due to horizontal DNA transfer with other pneumococci.

A complete MLVA profile was obtained for thirty-three serotype 5 pneumococci (figure 2). Nine distinct MLVA profiles were identified. Seven distinct MLVA profiles were associated with ST 289 (A–F, I) but all were closely related (>80% similarity). Type A matches the MLVA profile
of genotype 9 as reported in the MLVA database (http://www.mlva.eu). The two other MLVA profiles were associated with ST3468 (type G) and ST3844 (type H) and were more distant from types A–F. Type A seems to be well established in UK as it has accounted for 62% of all serotype 5 isolates tested since 2003 and was detected in all geographical regions where IPD caused by serotype 5 has been reported to RSIL. Six out of the 7 NE London cluster cases (case 1–6 and case 8) were identified as type B, providing strong evidence of the link between the case from the North–East of England and the other NE London cases. All of these cases were reported between 30/11/2007 and 11/01/2008. Type B has been identified in only one other serotype 5 isolate in the UK – in March 2005 from the South West region – and was not detected again until the cluster reported here. The re-emergence of this type in 2007 could either be a result of natural fluctuation in incidence of serotype 5 (7, 12); or due to serotype replacement following the introduction of 7-valent pneumococcal conjugate vaccine (PCV7) in September 2006 in the UK childhood vaccination scheme (see HPA website http://www.hpa.org.uk) or it could be due to importation from a country where serotype 5 is commonly associated with IPD and/or nasopharyngeal carriage. Case 7 (isolate no. 31), which was reported two months after the first outbreak of pneumonia was identified as type I.
Type I is genetically closer to type A than type B suggesting a different microbiological origin between case 7 and the December-January cluster. Therefore, case 7 should not be considered as belonging to the NE London outbreak. Two other cases of IPD (isolates no. 25 and 32, figure 2) caused by serotype 5 were reported during the same period of the NE London outbreak from two other hospitals located in different parts of London (Central and North East London areas). Both isolates were determined as ST289 and MLVA type A or D suggesting that, although these cases were reported during the same period, they were not linked to the NE London cluster.

Genetic characterization of pneumococci is essential to understand the epidemiology of IPD. By virtue of its ability to differentiate genotypes belonging to the same serotype and same ST, MLVA appears to be a valuable technique for outbreak investigation.

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Legends to figures

Figure 1: Epidemiological link between cases of serotype 5 paediatric pneumococcal pneumonia from North East London outbreak (December 2007 / March 2008)

Figure 2: Genetic relatedness of pneumococci of serotype 5 in UK (2003-2008)

Dendogram based on MLVA profile using UPGMA method.

Framed: pneumococci linked to North East London cluster.

* No PCR product visible on gel; ** R6: strain of reference for sizing tandem repeat; - : Missing data
Case 1 has an elder sibling who attends school and plays with case 2 but no known direct contact between case 1 and 2.

Case 1 has a younger sibling who attends kindergarten with a sibling of case 1.

Case 2 and 3 live on the same street.

Case 2 has a sibling who attends the same school as case 2.

Case 3 has older family members who attend the same place of worship as case 1.

Cases 5 and 6 belong to the same community as other cases and live in the same geographical area. But no definite links with other cases.

Case 8 lives in NE England but was visited by a relative from NE London community.
Isolates | Type | Profile | ST | Age (month) | Date | Region | Case no
--- | --- | --- | --- | --- | --- | --- | ---
1 | H | 7,9,4,1,-*,5,2 | 3844 | 6 | 2006-10-21 | Yorkshire & Humber |
2 | B | 7,8,2,1,2,9,4 | 289 | 34 | 2005-03-21 | South West |
3 | B | 7,8,2,1,2,9,4 | 289 | 30 | 2007-11-30 | London |
4 | B | 7,8,2,1,2,9,4 | 289 | 49 | 2007-11-30 | London |
5 | B | 7,8,2,1,2,9,4 | 289 | 86 | 2007-12-01 | London |
6 | B | 7,8,2,1,2,9,4 | 289 | 24 | 2007-12-27 | London |
7 | B | 7,8,2,1,2,9,4 | 289 | 53 | 2008-01-10 | London |
8 | B | 7,8,2,1,2,9,4 | 289 | 60 | 2008-01-11 | North East |
9 | A | 7,8,2,1,2,11,4 | 289 | - | 2003-12-31 | London |
10 | A | 7,8,2,1,2,11,4 | 289 | 0 | 2004-03-24 | London |
11 | A | 7,8,2,1,2,11,4 | 289 | 1 | 2004-03-13 | London |
12 | A | 7,8,2,1,2,11,4 | 289 | 1 | 2004-04-06 | London |
13 | A | 7,8,2,1,2,11,4 | 289 | 15 | 2003-02-24 | South East |
14 | A | 7,8,2,1,2,11,4 | 289 | 595 | 2005-06-02 | West Midlands |
15 | A | 7,8,2,1,2,11,4 | 289 | 546 | 2006-12-27 | South West |
16 | A | 7,8,2,1,2,11,4 | 289 | 731 | 2006-12-18 | London |
17 | A | 7,8,2,1,2,11,4 | 289 | 524 | 2007-01-16 | London |
18 | A | 7,8,2,1,2,11,4 | 289 | 805 | 2007-03-02 | Yorkshire & Humber |
19 | A | 7,8,2,1,2,11,4 | 289 | 537 | 2007-03-28 | Yorkshire & Humber |
20 | A | 7,8,2,1,2,11,4 | 289 | 251 | 2007-04-30 | London |
21 | A | 7,8,2,1,2,11,4 | 289 | 109 | 2007-10-06 | Yorkshire & Humber |
22 | A | 7,8,2,1,2,11,4 | 289 | 553 | 2007-10-22 | Yorkshire & Humber |
23 | A | 7,8,2,1,2,11,4 | 289 | 28 | 2007-10-23 | Yorkshire & Humber |
24 | A | 7,8,2,1,2,11,4 | 289 | 467 | - | West Midlands |
25 | A | 7,8,2,1,2,11,4 | 289 | 528 | 2007-11-28 | London |
26 | A | 7,8,2,1,2,11,4 | 289 | 2 | 2007-12-30 | Yorkshire & Humber |
27 | A | 7,8,2,1,2,11,4 | 289 | 1 | 2008-01-05 | Yorkshire & Humber |
28 | A | 7,8,2,1,2,11,4 | 289 | 64 | 2008-01-23 | Yorkshire & Humber |
29 | A | 7,8,2,1,2,11,4 | 289 | 22 | 2008-04-08 | Yorkshire & Humber |
30 | D | 7,-,2,1,2,11,4 | 289 | 10 | 2007-11-27 | London |
31 | I | 7,9,2,1,2,11,4 | 289 | 16 | 2008-03-12 | London |
32 | C | 7,8,2,1,2,11,4 | 289 | - | 2006-06-03 | Yorkshire & Humber |
33 | E | 7,8,2,1,4,11,4 | 289 | 28 | 2007-01-09 | Yorkshire & Humber |
34 | F | 7,8,1,1,2,11,4 | 289 | 31 | 2008-01-09 | Yorkshire & Humber |
35 | G | 7,8,1,1,11,7,4 | 3468 | 667 | 2007-01-04 | London |