Molecular Typing of Pneumococci for Investigation of Linked Cases of Invasive Pneumococcal Disease

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In winter 2007–2008, an outbreak of pediatric pneumonia caused by serotype 5 pneumococci was identified in a northeast London suburb. Variable number of tandem repeat analyses clustered these pneumococci from the other serotype 5 pneumococci in the United Kingdom, 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, septicemia, 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 localized 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 to 3) of pediatric 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 northeast (NE) 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 five more cases of clinical pneumonia in young children from this community. One of these was caused by serotype 3 pneumococcus and was therefore not included in the cluster. Case 4 was regarded as probable, as no sample was available for serotyping but the patient had close social links with case 2. Blood cultures from case 5 and case 6 yielded serotype 5 pneumococci. In case 7, a child with close social links to case 1 was admitted to the hospital with empyema in March 2008; direct detection of specific antigen (11) from a pleural fluid sample confirmed the diagnosis. 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 (Fig. 1). This cluster of serotype 5 IPD was recognized 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. Multilocus sequence typing analysis (MLST) and multilocus variable number of tandem repeat (VNTR) analysis (MLVA) were performed on five pneumococcal cultures (from cases 1, 2, 3, 5, and 6) and two DNA samples (from cases 7 and 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 ms39). 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 the R6 sequence. Clustering analyses were performed using the BioNumerics categorical coefficient and the unweighted-pair group method using average linkages...
MLST revealed that pneumococci of serotype 5 were genetically closely related. Out of 35 pneumococci, 32 shared the same allelic profile of sequence type (ST) 289. New STs were identified in two pneumococci: ST3468 and ST3844. ST3468 is a single-locus variant (SLV) of ST289. ST3844 is not related to the other United Kingdom isolates but is closely related to ST3590, previously reported in the 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 colonization of the nasopharynx is only transitory. Short periods of colonization 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 33 serotype 5 pneumococci (Fig. 2). Nine distinct MLVA profiles were identified. Seven distinct MLVA profiles were associated with ST289 (A to F and 1), 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 to F. Type A seems to be well established in the United Kingdom, 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 seven NE London cluster cases (cases 1 to 6 and case 8) were identified as type B, providing strong evidence of the link between the case from the NE of England and the other NE London cases. All of these cases were reported between 30 November 2007 and 11 January 2008. Type B has been identified in only one other serotype 5 isolate in the United Kingdom—in March 2005 from the southwest region—and was not detected again until the cluster reported here. The reemergence of this type in 2007 could be a result of either natural fluctuation in the incidence of serotype 5 (7, 12), serotype replacement following the introduction of the 7-valent pneumococcal conjugate vaccine (PCV7) in September 2006 in the United Kingdom childhood vaccination scheme (see HPA website [http://www.hpa.org.uk]), or importation from a country where serotype 5 is commonly associated with IPD and/or nasopharyngeal carriage. Case 7 (isolate no. 31 [Fig. 2]), which was reported 2 months after the first outbreak of pneumonia, was identified as type I. Type I is genetically closer to type A than to type B, suggesting different microbiological origins between case 7 and the December-January cluster. Therefore, case 7 should not be considered to belong to the NE London outbreak. Two other cases of IPD (isolates no. 25 and 32 [Fig. 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 northeast London areas). Both isolates were characterized 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 outbreak.
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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REFERENCES


