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- From genomes to tracing pathogens-

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Population structure of an outbreak-causing clonal group of *Streptococcus pneumoniae* in the United States as revealed by boxB minisatellite sequencing

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At least four outbreaks of invasive pneumococcal disease caused by serotype 12F, clonal complex 218 *Streptococcus pneumoniae* have occurred in the United States over the past 20 years. We studied the population structure of this clonal group using 199, mostly invasive, isolates from outbreaks and surveillance collected over 22 years from 33 US states and seven other countries. Conventional multilocus sequence typing identified five sequence types and could not distinguish between three of four outbreaks. To improve typing resolution, we developed a new sequence typing method based on 10 boxB minisatellite loci ascertained to be variable. This method identified 83 types and revealed that each outbreak was caused by a different type. Strong overall linkage disequilibrium among the boxB loci was detected, indicating a relatively clonal population. The observed boxB diversity was compared to that expected under three mutation models assuming mutation-drift equilibrium. While most loci best fit the infinite allele model, all of these neutral models were rejected for two loci including one locus adjacent to a putative group II intron that is possibly recombinant, and another locus adjacent to a putative amino acid transport system that is possibly under balancing selection. Surprisingly, phylogeographic analyses revealed two prominent sequence clusters that were geographically differentiated along a line that subdivides the eastern US from the western US. In addition, a cluster of Australian isolates and most other non-US isolates nested within the western cluster. The western cluster is inferred to be ancestral and reveals some interesting differences in diversity and linkage disequilibrium in comparison to the eastern cluster. To our knowledge, this is the first report of a phylogeographic pattern among pneumococci and may be detectable here because of the high invasive disease potential, involving rare carriage and transmission.