Molecular evolution and complete genome sequences in forensic analysis: *Neisseria gonorrhoeae* in a transmission case

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Abstract:

Molecular epidemiology and phylogenetic analyses are frequently used in the investigation of viral transmission cases in forensic contexts. Here, we present the methods and results of the analysis of a bacterial transmission in an alleged child abuse case using complete genome sequences obtained by high-throughput sequencing (HTS) methods.

Initial molecular microbiology analyses were performed at the hospital's Microbiology Service including the two case samples and five local controls. Multi-Locus Sequence Typing (MLST) could not distinguish between the suspect/victim and 3 control isolates (ST9363). Pulse Field Gel Electrophoresis (PFGE) was not able to distinguish between the suspect/victim and one of the local controls.

We obtained complete genome sequences of *Neisseria gonorrhoeae* from the victim, the suspect, and 29 unrelated controls of the same ST. 3 of these corresponded to the local controls and the rest were from different regions in Spain. We used HTS with Illumina technology (Nextera and NextSeq 150x2 paired-ends). The analysis of the genomes revealed that the victim and suspect isolates had identical sequences in both the bacterial chromosome and the single plasmid present in them. One of the local controls was very similar (differing in only 2 SNPs) to the case sequences, another was clearly different (64 SNPs) and the remaining controls were very divergent (1060–1661 SNPs). Additional cases of identity and very high similarity among controls were observed occasionally, pointing at recent transmission cases.

We evaluated the evidence in support of the suspect to victim transfer of the bacteria by computing the likelihood ratios (LR) of this and each of the alternative hypotheses. Each hypothesis corresponds to a phylogenetic tree in which the victim sequence conforms a monophyletic cluster with each of the local controls. The LR values obtained ranged from 22.34 up to 1280.16, thus providing strong statistical support for the transfer from the suspect to the victim of the sequenced *N. gonorrhoeae* isolates.

These results lead us to conclude that complete bacterial genome sequences obtained with HTS technologies may be a valuable tool for establishing recent transmission cases and, although more studies are needed, they have a great potential for being used in forensic analyses.