

Genomic assessment of within-host population variation in *Neisseria gonorrhoeae*: Implications for gonorrhoea transmission

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Background:

Mathematical modelling and genomic analyses are powerful methods for investigating transmission dynamics of *N. gonorrhoeae*; however often make the implicit assumption that *N. gonorrhoeae* isolates at different anatomical sites within the same individual are the same strain.

Methods:

In this study, we used two approaches to explore genetic diversity. First, we examined a collection of stored *N. gonorrhoeae* isolates sourced from multiple anatomical sites of single individuals attending a sexual health clinic in Melbourne from 2011-2019. Second, we obtained multiple colony picks from primary plates of clinical samples from individuals attending a sexual health clinic in Melbourne from 2019-2020. Whole genome sequencing and a variety of bioinformatics approaches were used to determine both within-host and within-sample genetic diversity.

Results:

Thirty-seven individuals were identified that had cultured *N. gonorrhoeae* from two or more anatomical sites (urogenital, anorectal, or oropharyngeal), with a final dataset of 105 isolates. In 35/37 (94.6%) individuals, infections were highly similar at the genetic level, with identical multi-locus sequence types (MLST) and multi-antigen sequence types (NG-MAST). Comparisons of isolates within each individual indicated that the maximum within-host pairwise SNP distance was 13 SNPs (median = 1, IQR: 0-3). Notably, four distinct multi-individual phylogenetic clusters were identified, where the maximum pairwise SNP distance was 19 SNPs (median = 6, IQR = 2-11). Similarly, comparisons of isolates within each sample indicated that the maximum within-sample pairwise SNP distance was 8 SNPs (median = 2, IQR:1-3).

Conclusion:

This study suggests that in most cases, the same strain of *N. gonorrhoeae* causes infection at multiple anatomical sites. However, WGS data alone cannot differentiate

between the same infecting strain or (re)infections from the same transmission network. These data guide recommendations regarding optimal bioinformatic approaches to infer genetic relatedness of *N. gonorrhoeae* and will help inform future studies of gonorrhoea transmission and epidemiology.

Disclosure of Interest Statement:

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