Material and methods

Gonococcal DNA was extracted using the Qiagen DNeasy Tissue Kit (Qiagen Inc., Valencia, California) according to the manufacturer's instructions. Genomes of the 25 isolates of *N. gonorrhoeae* were sequenced using an Illumina Hi-Seq platform according to established procedure and contigs were assembled using the CLC Genomics Workbench. However, no finishing, either by software or by PCR, was performed; the assemblies were left as sets of contigs. This meant, for example, that a contig might contain only a portion of a gene. The remainder of the gene might occur in another contig, or might be missing all together. The contig sets ranged in cardinality from 268 to 648 and N50 measure from 6186 to 39792. Illumina-derived contigs were mapped to a reference strain, FA1090 (GeneBank accession AE004969), using a UNIX/LINUX shell script and the EMBOSS suite programs needleall and est2genome. In all, 10 loci (*penA*, *abcZ*, *adk*, *gdh*, *glnA*, *gnd*, *fumC*, *pilA*, *pyrD* and *serC*) were extracted from each of the 25 the genome assemblies using additional scripts and occasional manual sequence editing. The regions of homologous recombinations within the housekeeping genes were identified using a non-parametric recombination detection method, SiScan (1), as earlier described (2). The DNA sequences were incorporated into molecular evolutionary analysis (MEGA) version 4 (3) for identification of SNPs. Phylogeny, based on SNPs, was inferred using the Minimum Evolution method (4). The Neighbor-joining algorithm (5) was used to generate the initial phylogenetic tree. The antimicrobial susceptibility of *N. gonorrhoeae* isolates to penicillin, tetracycline, spectinomycin, ciprofloxacin, cefixime, azithromycin and ceftriaxone was assessed, in duplicate, by the agar dilution method as described previously (6, 7). Evolutionary analysis, using STRUCTURE (8), was performed to evaluate the population structure of gonococcal strains and their evolutionary relationship using a combination of SNPs, antimicrobial susceptibility and *penA* alleles from the 25 strains. The programs CLUMPP (9) and DISTRUCT (10) were used to generate the initial graphs depicting the population structure of gonococcal strains.

References

1. **Gibbs MJ, Armstrong JS, Gibbs AJ.** 2000. Sister-Scanning: a Monte Carlo procedure for assessing signals in recombinant sequences. Bioinformatics **16:** 573-582.
2. **Vidovic S, Horsman GB, Liao M, Dillon JR.** 2011. Influence of conserved and hypervariable genetic markers on genotyping circulating strains of *Neisseria gonorrhoeae*. PLoS ONE. 6(12):e28259.doi:10.1371/journal.pone.0028259.
3. **Tamura K, Dudley J, Nei M, Kumar S.** 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol. **24:** 1596-1599.
4. **Rzhetsky A, Nei M.** 1992. A simple method for estimating and testing minimum evolution trees. Molecular Biology and Evolution **9:** 945-967.
5. **Saitou N, Nei M.** 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4:** 406-425.
6. **Clinical Laboratory Standards Institute.** 2006. M7-A7. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically – seventh edition: Wayne (PA): Clinical Laboratory Standards Institute.
7. **Thakur SD, Starnino S, Horsman GB, Levett PN. Dillon JR.** 2014. Unique combined *penA*/*mtrR*/*porB* mutations and NG-MAST strain types associated with ceftriaxone and cefixime MIC increases in a 'susceptible' *Neisseria gonorrhoeae* population. J. Antimicrob. Chemother. doi:10.1093/jac/dkt543.
8. **Pritchard JK, Stephens M, Donnelly P.** 2000. Inference of population structure using multilocus genotype data. Genetics **155:** 945-959.

9. **Jakobsson M, Rosenberg NA.** 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics **23:** 1801-1806.
10. **Rosenberg NA.** 2004. DISTRUCT: a program for the graphical display of population structure. Mol. Ecol. Notes 4: 137-138.