Within-host *Mycobacterium tuberculosis* diversity and its utility for inferences of transmission

Michael A. Martin, Robyn S. Lee, Lauren A. Cowley, Jennifer L. Gardy and William P. Hanage

**Abstract**

Whole genome sequencing in conjunction with traditional epidemiology has been used to reconstruct transmission networks of *Mycobacterium tuberculosis* during outbreaks. Given its low mutation rate, genetic diversity within *M. tuberculosis* outbreaks can be extremely limited—making it difficult to determine precisely who transmitted to whom. In addition to consensus SNPs (cSNPs), examining heterogeneous alleles (hSNPs) has been proposed to improve resolution. However, few studies have examined the potential biases in detecting these hSNPs. Here, we analysed genome sequence data from 25 specimens from British Columbia, Canada. Specimens were sequenced to a depth of 112–296×. We observed biases in read depth, base quality, strand distribution and read placement where possible hSNPs were initially identified, so we applied conservative filters to reduce false positives. Overall, there was phylogenetic concordance between the observed 2542 cSNP and 63 hSNP loci. Furthermore, we identified hSNPs shared exclusively by epidemiologically linked patients, supporting their use in transmission inferences. We conclude that hSNPs may add resolution to transmission networks, particularly where the overall genetic diversity is low.

**DATA SUMMARY**

1. Raw *M. tuberculosis* read files have been deposited in National Center for Biotechnology Information’s Sequence Read Archive (NCBI SRA) under accession number PRJNA413593 (Table S1, available in the online version of this article).
2. *M. tuberculosis* strain H37Rv is available from GenBank; accession number NC_000962.3.
3. *M. tuberculosis* strain CCDC5079 is available from GenBank; accession number CP001641.1.
4. Source code for processing of raw reads and variant calling is available from GitHub; https://github.com/c2-d2/within-host-diversity.

**INTRODUCTION**

*Mycobacterium tuberculosis* is the leading communicable cause of mortality worldwide, causing 1.7 million deaths in 2016 [1]. Whole genome sequencing (WGS) has been used to estimate the transmission network of *M. tuberculosis* outbreaks, with identical or highly similar genetic sequences providing support for direct transmission between patients [2–6]. However, *M. tuberculosis* is among the most homogeneous of bacterial species. Due to the low diversity of *M. tuberculosis* genomes, cases which are not epidemiologically linked can therefore have highly similar consensus sequences [7,8]. Within-host genetic diversity of *M. tuberculosis* has previously been described [9–12] and may be due to within-host evolution or co-infection. The analysis of this diversity, i.e. making use of polymorphisms that arise during infection that may be transmitted through non-stringent bottlenecks, has been proposed to increase resolution of transmission. Modelling studies suggest that overlooking this diversity can lead to erroneous transmission inferences using genomic data alone [13]. A number of tools have been proposed to incorporate within-host diversity into transmission...
estimates [14–18], but their application to real world data remains limited. Furthermore, methods to reliably identify within-host diversity from WGS data are not well established.

Here we present the analysis of WGS data from M. tuberculosis specimens with an emphasis on the identification of consensus SNPs (cSNPs) and heterogeneous alleles (hSNPs) indicative of within-host variation. While we highlight important sources of bioinformatics biases in the identification of hSNPs, we show that hSNPs may indeed provide additional resolution beyond the cSNP-based approach for inferences of transmission.

THEORY AND IMPLEMENTATION

Data collection

Samples were cultured at the British Columbia Centre for Disease Control Public Health Laboratory and clean sweeps of Lowenstein-Jensen (LJ) slants were sequenced at the BC Genome Sciences Centre using the Illumina HiSeq platform (Supplementary Methods) [2]. Epidemiological data (Table S1) were collected by local public health units as part of routine investigations and provided in non-nominal form. Included specimens were chosen from a larger study [19] to represent two clusters of cases defined by classical genotyping (Supplementary Methods). One of these was composed exclusively of locally transmitted cases, while the other included locally transmitted cases, epidemiologically unlinked cases arising from reactivation of tuberculosis infection acquired outside Canada, and serial specimens from a single patient.

Genomic investigation and definition of hSNPs

Reads were trimmed with Trimmmomatic v0.36 and aligned to the H37Rv reference genome (GenBank accession number: NC_000962.3) [20, 21] using BWA MEM v0.7.15 [22]. Local realignment was conducted using GATK v3.8.0 [23]. Identification of large sequence polymorphisms was used to assign lineage [24]. Variant calling and filtering was conducted using SAMtools v1.7 and BCFtools v1.7 [25]. Proline-glutamic acid (PE), proline-proline-glutamic acid (PPE) and proline-glutamic acid_polymorphic guanine-cytosine-rich sequence (PE_PGRS) (PE/PPE) genes were excluded, as the disproportionate number of variants in these regions was suggestive of mapping error (Fig. S1). Our complete analysis pipeline is shown in Fig. 1 and is described in the Supplementary Methods.

SNPs were considered consensus (cSNPs) if at least 90% of the reads supported the variant allele and hSNPs if greater than 10% and up to 90% of the reads supported the variant allele. SNPs with 10% or less of the reads supporting the variant allele were called as reference. Informative SNPs were defined as variants present in at least one but not all of the specimens. The possibility of co-infection with another lineage of M. tuberculosis was investigated by checking whether any hSNPs were present at lineage-defining alleles [26].

Impact Statement

Genomic analysis in outbreaks of pathogens such as Mycobacterium tuberculosis has allowed for more accurate estimation of transmission networks, aiding in control and response efforts. However, in instances when genetic diversity is low, analyses that overlook the variation within single hosts may be unable to resolve transmission. In this study, we investigate the application of such heterogeneous alleles (hSNPs) for inferring transmission. We identify critical sources of bias that need to be accounted for in the bioinformatics analysis when identifying these variants. In terms of transmission, many hSNPs identified were consistent with the consensus SNP-based approach and epidemiological data. hSNPs also provided genomic support for transmission between an epi-linked pair that would have been missed using consensus SNPs alone. Incorporating the analysis of hSNPs in future outbreaks may be important to help inform inferences of transmission.

Phylogenetic analysis

Concatenated SNP alignments were used as input for phylogenetic trees. hSNP alignments were generated using IUPAC ambiguity codes [27]. IQ-TREE v1.6.1 was used to infer maximum-likelihood (ML) phylogenies with ultrafast bootstrap support values as well as 100 random phylogenies [28–30]. Distance between trees was calculated using the Kendall–Colijn (KC) metric (lambda=0) [31, 32]. We also compared cSNP and hSNP phylogenies using the approximately unbiased (AU) test [33] in CONSEL v0.20 [34]. Further details are provided in the Supplementary Methods.

Alignment-induced bias in observed hSNPs

All specimens were confirmed to be M. tuberculosis complex and sequenced to at least 100 average depth [median (sd), range: 178 × (31.15), 112–296] (Table S2). Following initial filtering criteria [all reads with mapping quality (MQ) >30, average MQ>30, base quality (QUAL) >50, high-quality read depth (DP) >20], we identified 2945 SNP loci, where at least one specimen had a cSNP or hSNP identified (2573 cSNP and 167 loci were identified where at least one specimen had a cSNP and 167 loci were identified where at least one specimen had an hSNP; these are not mutually exclusive as cSNPs in one specimen could be hSNPs in another). After removing SNPs in PE/PPE regions, 2654 SNP loci, where at least one specimen had a cSNP or hSNP identified (2573 cSNP and 94 hSNP loci), remained. A narrower distribution of Phred-scaled stand bias (SP) scores was present amongst cSNPs as compared to hSNPs [mean (sd): 0.18 (1.28) versus 32.08 (30.74), respectively, Fig. S2]. We therefore additionally filtered for only variants with SP>60 (maximum observed cSNP SP: 58) and required >1 variant read on both strands.

Manual inspection of the alignments revealed that many positions initially called as hSNPs were probably due to
alignment error, where the alternative allele was almost exclusively on reads that had been soft-clipped during mapping (Fig. S3, Table S3). Consequently, we removed all reads with less than 100 aligned base pairs (representing 3.5% of reads with MQ >30 in all specimens) from our analysis. Seventeen loci had differential SNP calls following this filtering step; five loci which were erroneously called as hSNPs previously were subsequently called as reference and six loci which failed the filtering protocol before removing clipped reads were included following this additional step.

The final dataset included 2598 SNP loci, where at least one specimen had a cSNP or hSNP identified (2542 loci were identified where at least one specimen had a cSNP and 63 loci were identified where at least one specimen had an hSNP). cSNP distances ranged from 0 to 1237 (Table S4). hSNP bases were shared between one and 25 specimens (Fig. S4, Table S5). Specimens belonged to lineage 4 (Euro-American, n = 5) or lineage 2 (East-Asian, n = 20) (Table S1). There was no evidence of co-infection by multiple lineages of M. tuberculosis. Lineage 4 specimens were 672–675 cSNPs from H37Rv and lineage 2 specimens were 1196–1237 cSNPs from H37Rv. The number of observed hSNPs per specimen was correlated with the distance from H37Rv ($R^2$ =0.34); this suggests some inferred hSNPs may in fact be
generated by alignment errors, resulting from structural polymorphisms present in our specimens which are not in the reference genome. However, mapping the lineage 2 genomes to a lineage 2 reference did not significantly impact phylogenetic analysis or transmission inferences (Supplementary Results, Tables S6 and S7, Figs S5 and S6), consistent with a previous cSNP-based study [35].

A comparison of key quality metrics after filtering is shown in Table 1 and Fig. S7. On average, read depth was higher at observed hSNPs. Strand bias, base quality bias, mapping quality bias and read position bias were all more significant amongst hSNPs. Mapping quality was similar between cSNPs and hSNPs. Given the limited number of hSNPs amongst hSNPs, we assume an RPB value of 1.0 for SNPs with

|                      | cSNP          | hSNP          |
|----------------------|---------------|---------------|
| Mean                 | Mean          | Mean          |
| DP                   | 161.84        | 232.84        |
| QUAL                 | 228.00        | 209.13        |
| MQ                   | 59.71         | 59.42         |
| SP                   | 0.16          | 10.44         |
| BQB*                 | 0.98          | 0.68          |
| MQB*                 | 1.00          | 0.91          |
| RPB*                 | 0.98          | 0.28          |

*As BQB, MQB and RPB are only defined at positions with reference and variant reads, we assume an RPB value of 1.0 for SNPs with 100% variant reads

Also physogenetically clustered with high bootstrap P-values (Fig. 2).

Following transmission pairs (described in [36]), one had identical hSNPs (BC14-Mtb181 and BC14-Mtb263, cSNP distance: 1), while the other (BC12-Mtb044 and BC13-Mtb143, cSNP distance: 2) had an hSNP which was present in the 2012 specimen and present as a cSNP in the 2013 specimen, and three hSNPs present in the 2012 specimen were not observed in 2013. This may indicate a bottleneck during transmission, which would be consistent with the small infectious dose of M. tuberculosis [37].

Among the outbreak cluster involving four cases with pairwise cSNP distances between 0 and 1, there was a nearly identical pattern of hSNPs. The same hSNPs were also observed in the fifth lineage 4 specimen (BC12-Mtb107), which was 14–15 cSNPs from the outbreak cluster and not identified as being epidemiologically linked. This suggests that the observed hSNPs may be due to real underlying structural variation that is present within our specimens (and therefore epidemiologically relevant) but is not found in the reference, thereby resulting in alignment errors.

Our dataset also included four serial specimens collected between 2004 and 2007 (Table S1) from a single patient (‘Patient A’) diagnosed with multi-drug resistant (MDR) M. tuberculosis (Supplementary Results). These specimens differed from one another by a maximum of two cSNPs and 11 hSNPs. There was one hSNP present in the 2004 specimen that was called as reference in the remaining specimens. Two novel cSNPs, unique to these specimens, were observed in the 2006 specimens. One of these cSNPs was called as an hSNP in the 2007 specimen (82% variant read frequency); as this would have been missed by standard consensus-based SNP calling, this illustrates that hSNPs can provide additional information when determining genetic distance. Nine additional hSNPs were present in the 2007 specimen. While these may be due to within-host evolution, they may more likely be due to sampling; the bacteria in the previous sputum samples might not have been representative of the full range of diversity generated within the lung, a limitation of all genomic analysis of M. tuberculosis [38]. Alternatively, while there was no evidence of co-infection with a different M. tuberculosis lineage, co-infection with another closely related lineage 2 strain from outside our collection or laboratory cross-contamination [39] are also possible explanations. Otherwise, observed hSNPs and cSNPs were the same for all four specimens (Supplementary Results, Table S8, Fig. S9).

We also observed congruence in the phylogenetic signal present in hSNPs and cSNPs (Fig. 3). When we constrained a cSNP-based phylogeny to the topology of that created with hSNPs, the likelihood was −14 586.23, compared to −12 306.80 for a phylogeny defined only by cSNPs. Constraining the cSNP-based phylogeny to a set of randomly generated tree topologies resulted in much lower likelihood [mean (SD): −25 087.74 (450.46)]. Additionally, we found
the cSNP ML tree and the bootstrap replicates were congruent with the hSNP topology based on the AU test ($P>0.05$ for all comparisons). This suggests the phylogenetic signal provided by the hSNPs is much better than expected by chance. However, the KC distance was greater between cSNP and hSNP topologies (109.20) as compared to random [mean (SD): 68.44 (11.96)]. Furthermore, a tanglegram revealed differences between the cSNP and hSNP topologies, indicating that while hSNPs may provide additional information in the case of very closely related infections, they should be used as a complement to cSNP-based analyses and not independently for inferences of transmission.

**Conclusion**

We have used WGS data to identify within-host heterogeneity in *M. tuberculosis* amongst patients in British Columbia, Canada. Reliable methods to characterize within-host heterogeneity are needed to incorporate these data into epidemiological investigations. Our data included epidemiologically and/or genetically linked specimens from two *M. tuberculosis* lineages. We identified sources of bias leading to false positive identification of hSNPs, including excessively clipped reads, read depth, base quality, strand bias, base quality bias, mapping quality bias and read placement bias scores. We also observed concordance between hSNPs, cSNPs and epidemiological data. In one case, a shared variant was identified solely between two epidemiologically linked cases, BC12-Mtb044 and BC13-Mtb142; had hSNPs not been included in the analysis, this would have been missed. This affirms that the inclusion of hSNPs may provide additional resolution to inferences of transmission.

Ultra-deep sequencing may help to identify additional hSNPs and provide further discrimination between transmission events. Furthermore, long read sequencing may be useful in the identification of both cSNPs and hSNPs in repetitive regions of the genome. As suggested by Worby *et al.* [14], we expect hSNPs present in only a small number of very closely genetically related specimens to be the most informative; hSNPs found in a large number of unrelated infections probably reflect systematic analysis errors, or repeated mutation. Ultimately, the potential benefits of

---

**Fig. 2.** Maximum-likelihood (ML) phylogeny generated using an alignment of 2542 cSNPs, rooted on the H37Rv reference (REF). TVMe +ASC was identified as the best-fit model based on the Bayesian information criterion. Ultrafast bootstrap support values are annotated in blue (support values >95 % indicate high confidence) and epidemiological data provided by the BCCDC (British Columbia Centre for Disease Control) are indicated in the top left. Branches without annotation are not epidemiologically linked to other cases in the dataset. Each column at the right represents an informative hSNP as compared to H37Rv, ordered by position in the genome and coloured by supporting variant read frequency percentage. The scale bar corresponds to the number of substitutions per site.
this approach will be shown by applying the pipelines we describe here to larger datasets with low strain diversity.

While we have focused here predominantly on biases related to sequencing and bioinformatics approaches, it is important to recognize that all included samples were cultured prior to sequencing. There is currently limited knowledge about the impact of culture on heterogenous alleles. One study from 2017 compared sequencing from sputum with sequencing from MGIT culture using 17 paired samples and found that the median number of hSNPs was the same regardless of sequencing source [40]. However, another study with similar sample size found the opposite, with a significant loss of diversity when sequencing from MGIT culture [41]. Thus, it is possible that sequencing from culture influenced the hSNPs identified. Further investigation is needed on the impact of culture on detection of these heterogeneous alleles.

**Funding information**

This work was funded by an R01 grant from the National Institutes of Health, awarded to William Hanage (R01AI128344). Robyn Lee is also supported by a Fellowship from the Canadian Institutes of Health Research (MFE 152448). Epidemiological and genomic data collection was supported by the BCCDC Foundation for Population and Public Health, the Canada Research Chairs Program, and the Michael Smith Foundation for Health Research.

**Acknowledgements**

We would like to thank the team at the British Columbia Public Health Laboratory (Claire Kong, Mabel Rodrigues, Danielle Jorgensen and Inna Sekirov), BCCDC TB Services (James Johnston, Victoria Cook, David Roth and Fay Hutton), and UBC (Jennifer Guthrie) for providing the epidemiological and genomic data for this project. We would also like to thank Nathan Hicks of Sarah Fortune’s lab and Mohamad Sater of Yonatan Grad’s lab at the Harvard T.H. Chan School of Public Health for their advice on the analysis of WGS M. tuberculosis data.

**Conflicts of interest**

The authors declare that there are no conflicts of interest.

**Ethical statement**

All data were provided by the British Columbia Centre for Disease Control in non-nominal, de-identified form. Ethics approval for this project was obtained from the Harvard T.H. Chan School of Public Health Internal Review Board (IRB17-0802) and the University of British Columbia Internal Review Board (H12-00910). Individual patient consent was not required.

**Data bibliography**

DNA sequences have been deposited to NCBI SRA under accession number PRJNA413593 (Table S1). Additional sequences used in this study were also obtained from:
1. Camus JC, Pryor MJ, Médigue C, Cole ST. Re-annotation of the genome sequence of Mycobacterium tuberculosis H37Rv. Microbiology [Internet]. 2002;148(1350–1367):12967–73. Available from: pmid:12368430
2. Cole ST, Brosch R, Parkhill J, Garnier T, Churcher C et al. Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence. Nature 1998;393(6685):537–44.
3. Zhang Y, Chen C, Deng JLH, Pan A, Zhang L et al. Complete genome sequences of Mycobacterium tuberculosis strains CDC5079 and CDC5080, which belong to the Beijing family. J Bacteriol 2011;193 (19):5991–2.

References
1. WHO. Global tuberculosis report 2017. 2017.
2. Gardy JL, Johnston JC, Ho Sui SJ, Cook VJ, Shah L et al. Whole-genome sequencing and social-network analysis of a tuberculosis outbreak. N Engl J Med 2011;364:730–739.
3. Walker TM, Ip CL, Harrell RH, Evans JT, Kapatai G et al. Whole-genome sequencing to delineate Mycobacterium tuberculosis outbreaks: a retrospective observational study. Lancet Infect Dis 2013;13:1377–144.
4. Bjorn-Mortensen K, Soborg B, Koch A, Ladefoged K, Merker M et al. Tracking Mycobacterium tuberculosis transmission by whole genome sequencing in a high incidence setting: a retrospective population-based study in East Greenland. Sci Rep 2016;6:1–8.
5. Walker TM, Monk P, Smith EG, Peto T. Contact investigations for outbreaks of Mycobacterium tuberculosis: advances through whole genome sequencing. Clin Microbiol Infect 2013;19:796–802.
6. Stucki D, Balif M, Bodmer T, Coscolla M, Maurer AM et al. Tracking a tuberculosis outbreak over 21 years: strain-specific single-nucleotide polymorphism typing combined with targeted whole-genome sequencing. J Infect Dis 2015;211:1306–1316.
7. Lee RS, Radomski N, Proulx JF, Manry J, McIntosh F et al. Re-emergence and amplification of tuberculosis in the Canadian arctic. J Infect Dis 2015;211:1905–1914.
8. Lee RS, Radomski N, Proulx JF, Levade I, Shapiro BJ et al. Population genomics of Mycobacterium tuberculosis in the Inuit. Proc Natl Acad Sci USA 2015;112:13609–13614.
9. Gan M, Liu Q, Yang C, Gao Q, Luo T. Deep Whole-Genome Sequencing to Detect Mixed Infection of Mycobacterium tuberculosis. PLoS One 2011;6:e105929.
10. Black PA, de Vos M, Louw GE, van der Merwe RG, Dippenaar A et al. Whole genome sequencing reveals genomic heterogeneity and antibiotic purification in Mycobacterium tuberculosis isolates. BMC Genomics 2015;16:857.
11. Cohen T, Chindelevitch L, Misra R, Kempner ME, Galea J et al. Within-host heterogeneity of Mycobacterium tuberculosis infection is associated with poor early treatment response: a prospective cohort study. J Infect Dis 2016;213:1796–1799.
12. Trauner A, Liu Q, Via LE, Liu X, Ruan X et al. The within-host population dynamics of Mycobacterium tuberculosis vary with treatment efficacy. Genome Biol 2017;18:71.
13. Worby CJ, Lipsitch M, Hanage WP. Within-host bacterial diversity hinders accurate reconstruction of transmission networks from genomic distance data. PLoS Comput Biol 2014;10:e1003549.
14. Worby CJ, Lipsitch M, Hanage WP. Shared genomic variants: identification of transmission routes using pathogen deep-sequence data. Am J Epidemiol 2017;186:1209–1216.
15. De Maio N, Worby CJ, Wilson DJ, Stuessner N. Bayesian reconstruction of transmission within outbreaks using genomic variants. PLoS Comput Biol 2018;14:e1006117.
16. Wymant C, Hall M, Ratmann O, Bonsall D, Golubchik T et al. PHYLOSCANNER: inferring transmission from within- and between-host pathogen genetic diversity. Mol Biol Evol 2017;35:719–733.
17. Didelot X, Gardy J, Colijn C. Bayesian inference of infectious disease transmission from whole-genome sequence data. Mol Biol Evol 2014;31:1869–1879.
18. Didelot X, Fraser C, Gardy J, Colijn C. Genomic infectious disease epidemiology in partially sampled and ongoing outbreaks. Mol Biol Evol 2017;34:997–1007.
19. Guthrie JL, Kong C, Roth D, Jorgensen P, Rodrigues M et al. Molecular epidemiology of tuberculosis in British Columbia, Canada: a 10-year retrospective study. Clin Infect Dis 2018;66:849–856.
20. Cole ST, Brosch R, Parkhill J, Garnier T, Churcher C et al. Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence. Nature 1998;393:537–544.
21. Camus JC, Pryor MJ, Médigue C, Cole ST. Re-annotation of the genome sequence of Mycobacterium tuberculosis H37Rv. Microbiology [Internet]. 2002;148(1350–1367):12967–73. Available from: pmid:12368430
22. Col1 F, McNerney R, Guerra-Assunção JA, Glynn JR, Perdigão J et al. A robust SNP barcode for typing Mycobacterium tuberculosis complex strains. Nat Commun 2014;5:4.
23. Johnson AD. An extended IUPAC nomenclature code for polymorphic nucleic acids. Bioinformatics 2010;26:1386–1389.
24. Hoang DT, Chernomor O, van Haeseler A, de Jong BC et al. Variable host-pathogen compatibility in Mycobacterium tuberculosis. Proc Natl Acad Sci USA 2006;103:2869–2873.
25. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics 2009;25:2078–2079.
26. Kalyaanamoorthy S, Minh BQ, Wong TKF, Stamatakis A. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol 2015;32:268–274.
27. Kalyaanamoorthy S, Minh BQ, Wong TKF, Stamatakis A. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol 2015;32:268–274.
28. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics 2009;25:2078–2079.
29. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics 2009;25:2078–2079.
30. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics 2009;25:2078–2079.
31. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics 2009;25:2078–2079.
32. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics 2009;25:2078–2079.
33. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics 2009;25:2078–2079.
34. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics 2009;25:2078–2079.
35. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics 2009;25:2078–2079.
36. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics 2009;25:2078–2079.
37. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics 2009;25:2078–2079.
38. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics 2009;25:2078–2079.
39. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics 2009;25:2078–2079.
40. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics 2009;25:2078–2079.
41. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics 2009;25:2078–2079.
42. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics 2009;25:2078–2079.
43. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics 2009;25:2078–2079.
44. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics 2009;25:2078–2079.
39. Braden CR, Templeton GL, Stead WW, Bates JH, Cave MD et al. Retrospective detection of laboratory cross-contamination of *Mycobacterium tuberculosis* cultures with use of DNA fingerprint analysis. *Clin Infect Dis* 1997;24:35–40.

40. Votintseva AA, Bradley P, Pankhurst L, del Ojo Elias C, Loose M et al. Same-day diagnostic and surveillance data for tuberculosis via whole-genome sequencing of direct respiratory samples. *J Clin Microbiol* 2017;55:1285–1298.

41. Doyle RM, Burgess C, Williams R, Gorton R, Booth H et al. Direct whole-genome sequencing of sputum accurately identifies drug-resistant *Mycobacterium tuberculosis* faster than MGIT culture sequencing. *J Clin Microbiol* 2018;56.

---

**Five reasons to publish your next article with a Microbiology Society journal**

1. The Microbiology Society is a not-for-profit organization.
2. We offer fast and rigorous peer review – average time to first decision is 4–6 weeks.
3. Our journals have a global readership with subscriptions held in research institutions around the world.
4. 80% of our authors rate our submission process as ‘excellent’ or ‘very good’.
5. Your article will be published on an interactive journal platform with advanced metrics.

Find out more and submit your article at microbiologyresearch.org.