



HAL
open science

Accuracy of whole genome sequencing to determine recent tuberculosis transmission: an 11-year population-based study in Hamburg, Germany

Roland Diel, Thomas Kohl, Florian Maurer, Matthias Merker, Karen Meywald Walter, Jörg Hannemann, Albert Nienhaus, Philip Supply, Stefan Niemann

► To cite this version:

Roland Diel, Thomas Kohl, Florian Maurer, Matthias Merker, Karen Meywald Walter, et al.. Accuracy of whole genome sequencing to determine recent tuberculosis transmission: an 11-year population-based study in Hamburg, Germany. *European Respiratory Journal*, 2019, pp.1901154. 10.1183/13993003.01154-2019 . hal-02347059

HAL Id: hal-02347059

<https://hal.science/hal-02347059>

Submitted on 11 Dec 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Early View

Research letter

Accuracy of whole genome sequencing to determine recent tuberculosis transmission: an 11-year population-based study in Hamburg, Germany

Roland Diel, Thomas A. Kohl, Florian Mauer, Matthias Merker, Karen Meywald Walter, Jörg Hannemann, Albert Nienhaus, Philip Supply, Stefan Niemann

Please cite this article as: Diel R, Kohl TA, Mauer F, *et al.* Accuracy of whole genome sequencing to determine recent tuberculosis transmission: an 11-year population-based study in Hamburg, Germany. *Eur Respir J* 2019; in press (<https://doi.org/>).

This manuscript has recently been accepted for publication in the *European Respiratory Journal*. It is published here in its accepted form prior to copyediting and typesetting by our production team. After these production processes are complete and the authors have approved the resulting proofs, the article will move to the latest issue of the ERJ online.

Accuracy of whole genome sequencing to determine recent tuberculosis transmission: an 11-year population-based study in Hamburg, Germany

Roland Diel^{1,2*,&}, Thomas A. Kohl^{3,4,&}, Florian Mauer⁴, Matthias Merker^{3,4}, Karen Meywald Walter⁵, Jörg Hannemann⁵, Albert Nienhaus^{2,6}, Philip Supply^{7,8,9,10}, Stefan Niemann^{3,4}

¹Institute for Epidemiology, Schleswig-Holstein University Hospital, 24105 Kiel, Germany.

²Institution for Statutory Accident Insurance and Prevention in the Health and Welfare Services (BGW), Hamburg, Germany

³Molecular and Experimental Mycobacteriology, Research Center Borstel, 23845 Borstel, Germany.

⁴German Center for Infection Research, Partner Site Hamburg-Lübeck-Borstel-Riems, Borstel, Germany.

⁵Public Health Department Hamburg-Central, 20097 Hamburg, Germany.

⁶Institute for Health Services Research in Dermatology and Nursing, University Medical Center Hamburg-Eppendorf, Germany

⁷Univ. Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, U1019 - UMR 8204 - CIL - Centre d'Infection et d'Immunité de Lille, F-59000 Lille, France.

⁸ Centre National de la Recherche Scientifique (CNRS), Unité Mixte de Recherche (UMR) 8204, Center for Infection and Immunity of Lille, Lille, France.

⁹ Université Lille Nord de France, Center for Infection and Immunity of Lille, Lille, France.

¹⁰Institut Pasteur de Lille, Center for Infection and Immunity of Lille, Lille, France.

[&]equal contribution

*Correspondent footnote.

Roland Diel, Email: roland.diel@epi.uni-kiel.de

Main Text

Controlling human-to-human tuberculosis (TB) transmission is key for achieving the targets of the “End TB strategy” set by the World Health Organization [1, 2]. Stopping TB transmission in large cities especially is a challenging top-priority worldwide [3]. Metropolitan areas have higher TB case notification rates than the rest of the countries as they concentrate high risk groups, such as homeless, drug users, and migrants often from (other) high TB incidence settings. Opportunities for transmission are amplified by population density and complex social interactions, regularly leading to large, temporally extended transmission networks [3]. Targeted interventions to interrupt transmission require the combination of effective genotyping of TB strains with enhanced epidemiological investigation. Whilst classic IS6110 DNA fingerprinting and 24-locus-MIRU-VNTR (mycobacterial interspersed repetitive units-variable number of tandem repeat) typing provide standardized and easily computable typing results with an on-line nomenclature system, several studies have now demonstrated that whole genome sequencing (WGS) has a superior discriminatory power allowing for an unparalleled resolution of outbreak strains [4–10]. However, predictivity of WGS for detecting transmission in metropolitan areas has not yet been quantified versus most deterministic references, i.e. tangible epidemiological links identified by ad hoc investigation, at extended time and population scales.

To address this gap, we performed classical genotyping (IS6110 DNA fingerprinting and 24 locus MIRU-VNTR typing) and WGS based on Illumina technology, using established procedures [11–14], of *Mycobacterium tuberculosis* complex strains obtained over more than a decade (from 2005 and 2015) from 1171 patients living in Hamburg, Germany, representing 92.3% of all culture positive cases over the period. Patient strain clusters were defined based on identical classical genotyping patterns and a five-single nucleotide-polymorphism (SNP) genetic distance between any two strains as a cut-off for WGS (d5WGS) [15].

All 1171 patients were classified into “clustered” or “non-clustered” groups according to the respective results of IS6110 DNA fingerprinting, MIRU-VNTR typing and d5WGS. Clustering results were blindly matched against definite epidemiological links between patients, uninterruptedly identified over the period by systematic contact tracing including geographical mapping by the Public Health department. In Hamburg, data of all patients are collected prospectively by trained public health staff using a standardized questionnaire. Beyond capturing the identity of the patients’ household, occupational and social contacts, information is obtained on nationality, date and country of birth, immigration status (if necessary), date of entry to Germany, number of years of residency in Hamburg or elsewhere in Germany, present and prior address (es) in Hamburg or elsewhere for the last ten years (inclusive homeless shelters, if necessary), the nature of the patient's actual and prior employment(s), clinical diseases and any previous known exposure to other persons with TB (especially within the 6 months before development of any symptoms). These data improve identification of previously unaware transmissions which may be clarified in additional interviews. In addition, they also help to confirm lacking epidemiological links, e.g., if two cluster members had been infected by a highly prevalent regional strain in their respective home country before “importing” it to Hamburg, but had never met each other.

We defined the sensitivity of a method as the fraction of TB patients clustered by this method that had epidemiologically confirmed transmission links among all epidemiologically linked patients. Specificity was defined as the fraction of patients not clustered, without identified epidemiological

link, among all (clustered and not clustered) patients without transmission. The positive predictive value (PPV) was the percentage of clustered patients who actually had an epidemiologically confirmed transmission among all clustered patients, whereas the negative predictive value (NPV) was the percentage of patients not clustered among those without an epidemiologically confirmed transmission.

From 1 January 2005 to 31 December 2015, cultures from 1171 patients' isolates, including 904 (77.2%) with pulmonary and 267 with extrapulmonary TB (22.8%), were available for investigation by the 3 genotyping methods. The average patient age was 46.0 ± 19.4 years (mean \pm SD), with 60.3% male (706/1171). 744 patients (63.5%) were foreign-born, coming from 96 different countries. For a total of 135 of the 1171 patients (11.5%) epidemiologically confirmed links could be identified (Table 1).

WGS analysis grouped 351 of the 1171 patients (31.9%) into 87 WGS clusters comprising two to 25 subjects (Table 1). In 35 WGS clusters, no detectable transmission links were found between any cluster members (40.2%), whilst in 52 clusters (59.8%) there was at least one detected transmission link between two cluster members (data not shown). These 52 clusters included 134 of the 135 individuals for whom a definite epidemiological link had been found. Only one single patient involved in a recent transmission chain was thus not captured and falsely classified as not clustered by d5WGS resulting in a sensitivity of 99.3% [95% CI: 95.9-100%] (134/135) (Table 1). As no transmission event could be detected in 217 (351-134) of the WGS-clustered patients, as for 819 of the non-clustered patients, specificity was 79.1% [95% CI 76.4-81.5%] (819/819+217). The PPV of d5WGS for detecting tangible epidemiological links between patients was 134/351, or 38.2% [95% CI 33.1-43.5%]. Conversely, the NPV was 99.9% [95% CI 99.2-100%], with 819 patients not clustered among 820 without evidence for any transmission link.

IS6110 DNA fingerprinting and MIRU-VNTR typing both assigned 131 of the 135 epidemiologically linked patients in our total set of 1171 patients as cluster patients, thus each achieving a sensitivity of 97.0% [95% CI 94.2-99.9%]. (Table 1)

However, MIRU-VNTR typing assigned 471 patients to clusters, including 131 of the 135 patients with a definite epidemiological link (Table 1). Conversely, 696 of the remaining 700 patients, who were not assigned to a cluster, had no epidemiological link. Thus, the specificity for detection of recent transmission chains was 67.2% (696/340+696) [95%CI 64.3-70.0%], i.e. 11.9% lower than WGS. Compared to WGS, the PPV of the MIRU was also substantially lower with 131/471, or 27.8% [95% CI 23.8-31.9%] whilst the NPV was nearly identical with 99.4% (696/700) [95% CI 98.4-100%].

IS6110 genotyping performed slightly better, with clusters comprising 417 patients in total, thus 54 less than with MIRU-VNTR typing (Table 1). Consequently, specificity was 750/(286+750), or 72.40% [95% CI 69.7-75.1%]. The PPV of the IS6110 genotyping method was 131/417, or 31.4% [95% CI 27.0-35.9%], whilst the NPV was 99.5% (750/754) [95% CI 99.0-100%].

Our results are consistent with the findings published in Nikolayevskyy's most recently published review [16] demonstrating that a cut off fewer than 6 SNPs is key to discriminate between TB patients with suspected transmission and those who are not. However, with respect to the lower specificity of 74% of the d5WGS method for detecting cluster patients with confirmed epidemiological links (compared to its excellent sensitivity of nearly 100%), the results of our study suggest that even a cut-off of 5 SNPs alone, i.e. without additional epidemiological information, is

not able to fully discriminate between those cluster members with verified transmission and those without, e.g., appearing in a cluster by coincidence, but without person-to-person transmission.

Our results are also consistent with findings from a 4-year study of a patient population of the English Midlands, which showed that the expected positive association between a risk factor such as geographic proximity to another TB case and WGS-based clustering vanished with cut-offs exceeding 5 SNPs [8]. The latter study also found that MIRU-VNTR clusters, combined with shared risk factors between patients, positively predicted only half of 5-SNP-based WGS clusters, taken as a “self-defining” reference of recent transmission. Here, we used deterministic links established by intensive epidemiological investigation as an external reference, to determine PPVs and NPVs of both WGS and classical methods independently to detect transmission.

As a limitation, our epidemiological investigation likely missed a number of less tractable epidemiological links (e.g. casual contacts). However, the subtle and prospectively performed sociodemographic in-depth analysis of all TB patients minimized such misclassifications by increasing both, sensitivity and specificity. Furthermore, while missed links would cause an underestimation of the PPVs of the respective genotyping methods, this should affect all methods equally.

Compared to classical fingerprinting, genotyping with d5 WGS is generally able to more precisely define target groups for health examinations and to show how contact tracing strategies could be adapted to population subgroups with a high potential of exposure to *M. tuberculosis* transmission. This may, for example, be of help in larger ongoing TB outbreaks in which long-term spreads of a single MTB-strain may occur over decades in the same milieu. Here, typing with WGS has been demonstrated to better assign indexes case to recently infected, “true” secondary cases than genotyping with IS6110 RFLP and thus may speed up the start of more focused contact investigations [6].

In addition to more precise transmission analysis, prospective use of rapid WGS also results in clinical benefits for the patients as resistances can be rapidly determined from WGS data and used for guiding individualized treatment [1, 9].

For instance, only one initial patient in each of 3 two-person clusters based on IS6110 fingerprinting and MIRU-VNTR typing had an INH-resistance. Each of these pairs were ungrouped by WGS (data not shown), which avoided an unnecessary modification of TB therapy for each second cluster patient who in fact turned out to have fully susceptible TB once phenotypic drug susceptibility (DST) results were obtained. Likewise, only 3 of 6 multidrug resistant TB patients in one single Beijing-type MIRU-VNTR cluster had resistance to all oral second line drugs, while the other 3 showed susceptibility to prothionamide and moxifloxacin, raising uncertainty on which resistance patterns were correctly identified. By d5WGS, those patients were split up into two different MDR-TB clusters and the contact persons of the index cases in the second cluster could receive a personalized prophylactic combination of prothionamide and moxifloxacin (data not shown).

Especially in MDR-/XDR-TB patients, the rapid initiation of effective treatment regimens already weeks prior to receiving the lengthy phenotypic DST results will avoid ongoing transmission. Actually, the scientific evidence suggests that WGS resistance predictions have reached a precision allowing their clinical use and can potentially replace phenotypic DST for first line drugs [17]. This will likely be the case for second line and new drugs in the future.

In conclusion, WGS typing with a 5-SNP cutoff delineates recent transmission chains with high accuracy and also provides high resolution resistance patterns, thus, enabling direct clinical benefits. Thus, WGS typing, especially conducted in metropolitan areas, may highly effectively strengthen national contact investigation policies. As the WHO End TB Strategy promotes the early diagnosis of all cases and active case finding, WGS fingerprinting should routinely be incorporated in national TB programmes, at least in those of high-income European countries.

Acknowledgements

We would like to thank I Radizio, Vanessa Mohr, T. Ubben and J. Zallet from Borstel, Germany for technical assistance, the staff from the public health offices in Hamburg and C. Allix-Béguet, C.Gaudin, M. Mairey, and S. Duthoy from Genoscreen for their help in genome sequencing. Parts of this work have been supported by the European Union PathoNgenTrace (FP7-278864) project, Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany's Excellence Strategy – EXC 22167-390884018, Leibniz Science Campus EvoLUNG, and the German Center for Infection Research. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. Raw sequence data (fastq files) have been used from published datasets deposited at the deposited at the European Nucleotide Archive (ENA) [7].

References

1. Dheda K, Gumbo T, Maartens G, Dooley KE, McNerney R, Murray M, Furin J, Nardell EA, London L, Lessem E, Theron G, van Helden P, Niemann S, Merker M, Dowdy D, Van Rie A, Siu GKH, Pasipanodya JG, Rodrigues C, Clark TG, Sirgel FA, Esmail A, Lin H-H, Atre SR, Schaaf HS, Chang KC, Lange C, Nahid P, Udwadia ZF, Horsburgh CR, et al. The epidemiology, pathogenesis, transmission, diagnosis, and management of multidrug-resistant, extensively drug-resistant, and incurable tuberculosis. *The Lancet Respiratory Medicine* 2017; 5: 291–360.
2. World Health Organisation (WHO). End TB Strategy. Available from: https://www.who.int/tb/post2015_strategy/en. Date last accessed 5 August 2019
3. van Hest NA, Aldridge RW, de Vries G, Sandgren A, Hauer B, Hayward A, Arrazola de Oñate W, Haas W, Codecasa LR, Caylà JA, Story A, Antoine D, Gori A, Quabeck L, Jonsson J, Wanlin M, Orcau Å, Rodes A, Dedicoat M, Antoun F, van Deutekom H, Keizer S, Abubakar I. Tuberculosis control in big cities and urban risk groups in the European Union: a consensus statement. *Euro Surveill* 2014; 19.
4. Bjorn-Mortensen K, Soborg B, Koch A, Ladefoged K, Merker M, Lillebaek T, Andersen AB, Niemann S, Kohl TA. Tracing Mycobacterium tuberculosis transmission by whole genome sequencing in a high incidence setting: a retrospective population-based study in East Greenland. *Scientific Reports* [Internet] 2016 [cited 2017 Sep 21]; 6 Available from: <http://www.nature.com/articles/srep33180>.
5. Lee RS, Radomski N, Proulx J-F, Manry J, McIntosh F, Desjardins F, Soualhine H, Domenech P, Reed MB, Menzies D, Behr MA. Reemergence and Amplification of Tuberculosis in the Canadian Arctic. *Journal of Infectious Diseases* 2015; 211: 1905–1914.
6. Roetzer A, Diel R, Kohl TA, Rückert C, Nübel U, Blom J, Wirth T, Jaenicke S, Schuback S, Rüsck-Gerdes S, Supply P, Kalinowski J, Niemann S. Whole Genome Sequencing versus Traditional Genotyping for Investigation of a Mycobacterium tuberculosis Outbreak: A Longitudinal Molecular Epidemiological Study. Neyrolles O, editor. *PLoS Medicine* 2013; 10: e1001387.
7. Walker TM, Merker M, Knoblauch AM, Helbling P, Schoch OD, van der Werf MJ, Kranzer K, Fiebig L, Kröger S, Haas W, Hoffmann H, Indra A, Egli A, Cirillo DM, Robert J, Rogers TR, Groenheit R, Mengshoel AT, Mathys V, Haanperä M, Soolingen D van, Niemann S, Böttger EC, Keller PM, Avsar K, Bauer C, Bernasconi E, Borroni E, Brusin S, Coscollá Dévis M, et al. A cluster of multidrug-resistant Mycobacterium tuberculosis among patients arriving in Europe from the Horn of Africa: a molecular epidemiological study. *The Lancet Infectious Diseases* 2018; 18: 431–440.
8. Wyllie DH, Davidson JA, Grace Smith E, Rathod P, Crook DW, Peto TEA, Robinson E, Walker T, Campbell C. A Quantitative Evaluation of MIRU-VNTR Typing Against Whole-Genome Sequencing for Identifying Mycobacterium tuberculosis Transmission: A Prospective Observational Cohort Study. *EBioMedicine* 2018; 34: 122–130.
9. Walker TM, Kohl TA, Omar SV, Hedge J, Del Ojo Elias C, Bradley P, Iqbal Z, Feuerriegel S, Niehaus KE, Wilson DJ, Clifton DA, Kapatai G, Ip CLC, Bowden R, Drobniewski FA, Allix-

- Béguet C, Gaudin C, Parkhill J, Diel R, Supply P, Crook DW, Smith EG, Walker AS, Ismail N, Niemann S, Peto TEA. Whole-genome sequencing for prediction of Mycobacterium tuberculosis drug susceptibility and resistance: a retrospective cohort study. *The Lancet Infectious Diseases* 2015; 15: 1193–1202.
10. Merker M, Kohl TA, Niemann S, Supply P. The Evolution of Strain Typing in the Mycobacterium tuberculosis Complex. *Adv. Exp. Med. Biol.* 2017; 1019: 43–78.
 11. van Embden JD, Cave MD, Crawford JT, Dale JW, Eisenach KD, Gicquel B, Hermans P, Martin C, McAdam R, Shinnick TM. Strain identification of Mycobacterium tuberculosis by DNA fingerprinting: recommendations for a standardized methodology. *J. Clin. Microbiol* 1993; 31: 406–409.
 12. Supply P, Lesjean S, Savine E, Kremer K, van Soolingen D, Loch C. Automated high-throughput genotyping for study of global epidemiology of Mycobacterium tuberculosis based on mycobacterial interspersed repetitive units. *J. Clin. Microbiol.* 2001; 39: 3563–3571.
 13. Merker M, Barbier M, Cox H, Rasigade J-P, Feuerriegel S, Kohl TA, Diel R, Borrell S, Gagneux S, Nikolayevskyy V, Andres S, Nübel U, Supply P, Wirth T, Niemann S. Compensatory evolution drives multidrug-resistant tuberculosis in Central Asia. *eLife* [Internet] 2018 [cited 2019 Mar 27]; 7 Available from: <https://elifesciences.org/articles/38200>.
 14. Kohl TA, Utpatel C, Schleusener V, De Filippo MR, Beckert P, Cirillo DM, Niemann S. MTBseq: a comprehensive pipeline for whole genome sequence analysis of Mycobacterium tuberculosis complex isolates. *PeerJ* 2018; 6: e5895.
 15. Walker TM, Merker M, Kohl TA, Crook DW, Niemann S, Peto TEA. Whole genome sequencing for M/XDR tuberculosis surveillance and for resistance testing. *Clinical Microbiology and Infection* 2017; 23: 161–166.
 16. Nikolayevskyy V, Niemann S, Anthony R, et al. Role and value of whole genome sequencing in studying tuberculosis transmission. *Clin Microbiol Infect* 2019 Apr 11.
 17. Meehan CJ, Goig GA, Kohl TA, Verboven L, Dippenaar A, Ezewudo M, Farhat MR, Guthrie JL, Laukens K, Miotto P, Ofori-Anyinam B, Dreyer V, Supply P, Suresh A, Utpatel C, van Soolingen D, Zhou Y, Ashton PM, Brites D, Cabibbe AM, de Jong BC, de Vos M, Menardo F, Gagneux S, Gao Q, Heupink TH, Liu Q, Loiseau C, Rigouts L, Rodwell TC, Tagliani E, Walker TM, Warren RM, Zhao Y, Zignol M, Schito M, Gardy J, Cirillo DM, Niemann S, Comas I, Van Rie A. *Nat Rev Microbiol.* 2019 Jun 17.

Table 1. Comparison of the three genotyping methods with respect to predicting MTB transmission by clustering

(Total number of patients = 1171, of those 135 patients with a confirmed epidemiological link)

	Total number of generated clusters	Total number of patients in clusters	Cluster patients with epidemiological link	Sensitivity [†]	Specificity [‡]	PPV [¶]	NPV [‡]	Accuracy [‡]
d5WGS	87	351	134	99.3% [95% CI 95.9-100%]	79.1% [95% CI 76.4-81.5%]	38.2% [95% CI 33.1-43.5%]	99.9% [95% CI 99.2-100%]	81.2% [95% CI 78.9-83.4%]
MIRU-VNTR	131	471	131	97.0% [95% CI 94.2-99.9%]	67.2% [95% CI 64.3-70.0%]	27.8% [95%CI23.8-31.9%]	99.4% [95% CI 98.9-100%]	70.6% [95% CI 68.0-73.2%]
IS6110 DNA Fingerprint	110	417	131	97.0% [95% CI 94.2-99.9%]	72.4% [95% CI 69.7-75.1%]	31.4% [95% CI 27.0-35.9%]	99.5% [95% CI99.0-100%]	75.2 [95% CI 72.8-77.7%]

[†]Percentage of cluster patients with a confirmed link among all patients with a confirmed transmission link[‡]Percentage of unclustered patients among all patients without confirmed transmission[¶]Percentage of clustered patients with a confirmed transmission among all clustered patients[‡]Percentage of patients assigned as unclustered among those without a confirmed transmission[‡](True Positives +True Negatives)/ (True Positives + False Positives + True Negatives + False Negatives)