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Early View

Research letter

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

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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 IS*6110* 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 IS*6110* 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 ± 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% C: 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.

IS*6110* 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%).

IS*6110* 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 IS*6110* 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 IS*6110* 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 protionamide 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.

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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[ŧ]	Specificity[t]	PPV[p]	NPV[ǂ]	Accuracy[j]
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%]

[ŧ]Percentage of cluster patients with a confirmed link among all patients with a confirmed transmission link

[t]Percentage of unclustered patients among all patients without confirmed transmission

[p]Percentage of clustered patients with a confirmed transmission among all clustered patients

[ǂ]Percentage of patients assigned as unclustered among those without a confirmed transmission

[j](True Positives +True Negatives)/ (True Positives + False Positives + True Negatives + False Negatives)