Routine survey of *Mycobacterium tuberculosis* isolates reveals nosocomial transmission

To the Editor:

Control of *Mycobacterium tuberculosis* transmission in high-income healthcare settings and in low tuberculosis (TB) prevalence countries remains a public health priority given the constant changes in *M. tuberculosis* epidemiology worldwide. Though Europe is a low prevalence area [1], TB burden among precarious and migrant populations contributes to this evolving landscape, as addressed by the action framework towards TB elimination [2]. At the core of the national healthcare system, tertiary care hospitals manage both patients with greater susceptibility to TB, and patients with complex and/or advanced TB disease. Key measures for TB control rely on enabling linkage of cases and identification of transmission chains, often supported by molecular survey tools [3, 4]. This is achieved by highlighting matched genotypes through a population-based systematic molecular TB survey in order to uncover outbreaks, even between apparently unrelated cases [5]. On that basis, further investigations can be triggered to understand the circumstances of transmission. In case of healthcare-related transmission, the origin of TB exposure is difficult to track as clinical disease develops months or years after patient discharge [6]. Therefore, understanding nosocomial TB transmission is essential for implementing control measures preventing such events. Herein, we bring evidence of the usefulness of molecular survey programmes to detect unsuspected TB transmission events among highly susceptible populations, and from a multidrug-resistant (MDR)-TB patient to a community-based individual. Subsequent corrective interventions implemented to prevent further nosocomial TB transmission are also described.

Since 2008, the laboratory of the Lyon University Hospital has implemented a prospective population-based survey by systematic genotyping of *M. tuberculosis* isolates from the surrounding geographic districts (ORAM: *Observatoire Rhône-Alpes des Mycobactéries*), to facilitate the reporting of clinical microbiology results from TB laboratories to an operational network including TB management and control centres, and a centralised regional health agency [7]. In addition to spoligo- and MIRU-VNTR15-typing performed as described [7], from 2016 onwards, whole genome sequencing (WGS) was implemented as previously reported [5]. Sampling and testing were performed upon diagnosis and in accordance with the French bioethics laws. Contact-tracing was conducted as recommended by the French guidelines [8].

From 2008 to 2018, 3230 cases of microbiologically proven TB were recorded; 20.5% of these cases were clustered, representing 662 patients distributed in 217 clusters. Among 96 clusters (341 cases) with confirmed transmission (patients knew each other), four were healthcare-related (transmission occurring in hospital area), representing eight patients, including a single MDR-TB transmission event (figure 1a). Among them, three cases in lung transplant recipients resulted from nosocomial transmission of drug-susceptible TB. All index cases were men aged from 44 to 72 years, admitted for COPD for which air-borne prevention measures were not applied, as TB was not primarily suspected. Only one index patient was smear positive for acid-fast bacilli examination. The secondary cases were women aged of 24 to 42 years, lung-transplanted for cystic fibrosis or idiopathic pulmonary fibrosis. TB was diagnosed between 4 and 12 months post-transplant, resulting in a related fatality case for one patient. The genomic analysis of the causal *M. tuberculosis* strain of each secondary case revealed a similar genotype (same spoligo- and MIRU-VNTR15-type) to the isolate obtained from the respiratory samples of the

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Tuberculosis is the number one life-threatening contagious disease. Ongoing systematic and prospective *M. tuberculosis* genotyping enables tracing transmissions, enhances active case finding and allows implementation of effective airborne control measures. http://bit.ly/2OyJvKx

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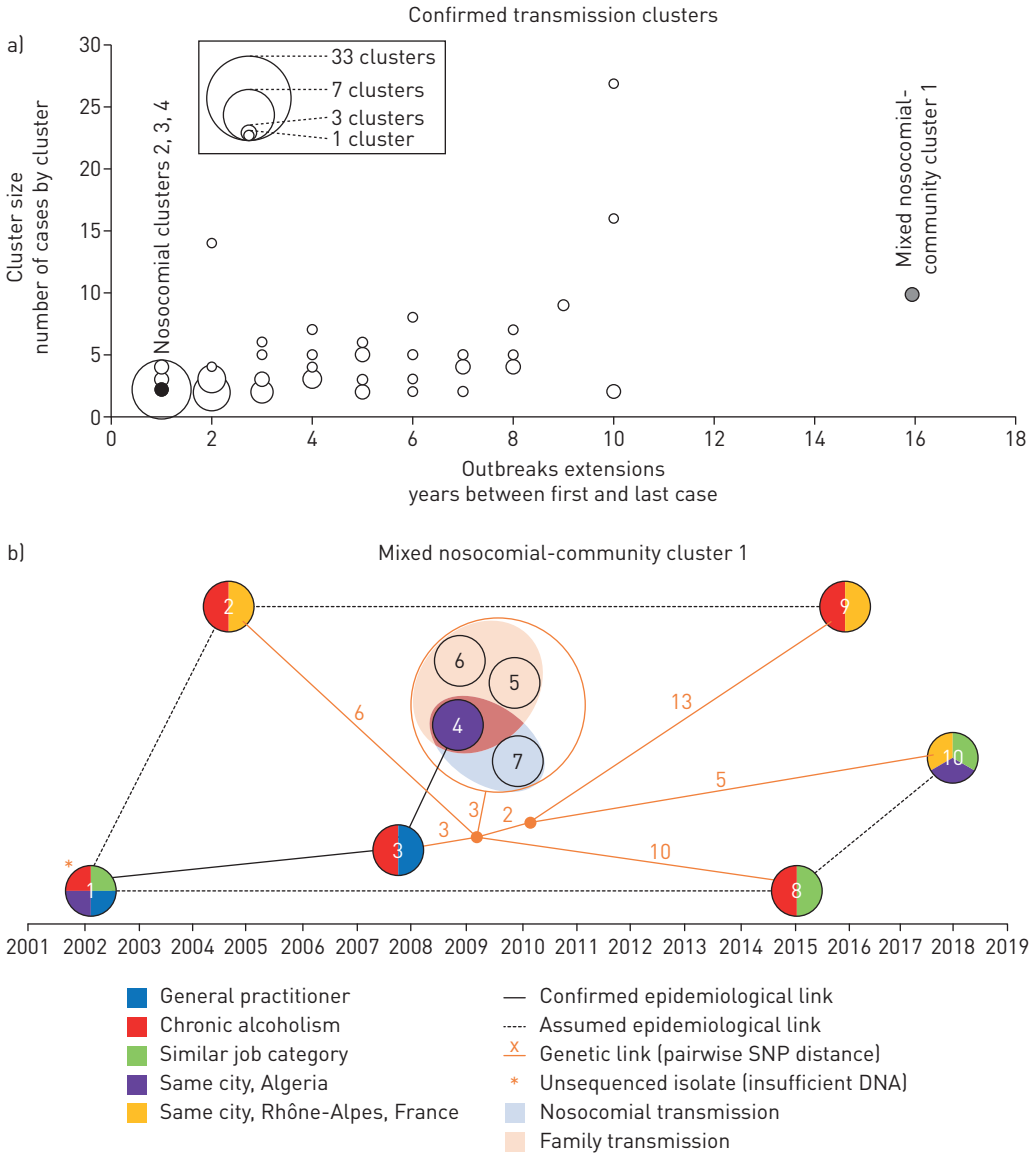

FIGURE 1 Size and time persistence of confirmed transmission clusters and detailed transmission chains within a unique cluster. a) Ball graph of 96 confirmed transmission clusters (patients knew each other, representing 341 patients) carrying common characteristics (size and persistence). The surface of the balls represents the number of clusters with common size and duration characteristics. White clusters are community-related and black clusters are nosocomial-related. The grey cluster contains both nosocomial- and community-related cases. b) Summary of the epidemiological links and genetic distances between the 10 chronological case-patients, over a 16-year period, of the grey cluster above. Circle colours indicate potential epidemiological links between patients and black lines the kind of epidemiological links established. Confirmed transmission: patients knew each other; assumed transmission: patients did not know each other but shared origins, habits, neighbourhood or locations in which transmission may have possibly occurred. Orange lines represent genetic distance between *Mycobacterium tuberculosis* isolates. Case 4 is the index case in nosocomial transmission and case 7 is the secondary case. Cases 5 and 6 were household-related with this index case. Cases 3 and 4 possibly met in 2008 due to frequent hospital admissions for the first and frequent business visits in the same hospital for the second. Cases 1 and 3 shared the same general practitioner, neighbourhood and life conditions (social care housing and chronic alcoholism). Cases 8 and 10 could have met for short periods owing to having similar jobs. Cases 1, 2, 3, 8 and 9 suffered from chronic alcoholism, implying a possibility of occasional meetings in bars. Cases 1, 4, and 10 were linked to the same city in Algeria.

corresponding index case. As pairs represented unique genotypes in the survey database, nosocomial transmission was therefore suspected and reported to the infection control unit; WGS performed later showed none or a single SNP pairwise distance for each couple of isolates. Thus, genotyping provided evidence of *de novo* transmission rather than donor-derived or endogenous reactivation [9]. Refined investigation of the infection control unit revealed possible transmission upon brief contacts before

transplantation in the collective areas of the hospital for two events, and at 3 months post-transplant, in the waiting room of the department for the third event. Though TB disease development risk is enhanced by repeated exposures to contagious patients [10], a certain degree of immune deficiency and/or some strain ability to efficiently infect a host may still favour TB infection upon brief exposure to *M. tuberculosis* aerosols [11].

The fourth nosocomial transmission event occurred between a 29-year-old man from Georgia requesting treatment for MDR-TB and a 23-year-old man without TB risk factors but living 400 m away from the hospital. Though placed in a negative-pressure ventilation room, the index case repeatedly broke isolation measures, going outside the building of the hospital. The *M. tuberculosis* isolate cultured from the secondary case's lung biopsy displayed spoligotype, MIRU-VNTR profile and a set of resistance mutations identical to the isolate of the MDR-TB patient from Georgia admitted 2 years earlier. WGS confirmed these findings (only four SNP distance between the two isolates). It should be noted that the causal *M. tuberculosis* strain was a member of the successful MDR Beijing clone W148, previously reported as highly transmissible [12].

Our surveillance detected a unique cluster of close-related strains isolated over a 16-year period and responsible for cases both in the hospital- and community-based settings (figure 1b). One of the lung transplant recipient TB cases belongs to this cluster, including, besides the index case, two household-related cases, and other cases possibly linked through different settings (same general practitioner, neighbourhood and life conditions, such as social care housing and chronic alcoholism). Three patients from the cluster were particularly related to the same location in Algeria. Successive strain importations from one country to another may explain the resurgence of the genotype over time. This hypothesis is supported by the high genetic distance, up to 18 SNPs between certain strains of the cluster.

Four nosocomial TB transmissions have been detected thanks to a real-time molecular survey of *M. tuberculosis* isolates. Of utmost importance, this has allowed prompt control measures to prevent additional similar events. The first active control measure was to optimise the population-based surveillance through WGS genotyping of all TB strains isolated in the Rhône-Alpes region coupled to real-time comparison of strains, as part of routine TB diagnosis. Transmission assumptions based on isolate genotyping are reported directly to the TB control units managing the territories where the identified cases live, in order to prompt epidemiological investigation. Detection of any unknown transmission chain is notified to the regional public health agency to assess if particular prevention measures should be implemented and to further relay the information to other regions possibly involved in the contact-tracing. This experience highlights the pivotal role of regional reference laboratories such as ORAM performing both diagnosis and survey to rapidly redirect information towards a TB network for effective decision-making and intervention. Numerous studies showed the potential of WGS to more accurately quantify transmission of both MDR-TB and drug-susceptible TB [13], and new strategies are emerging to decipher transmission events [4]. However, at the moment, few studies have been conducted prospectively with implementation of prevention measures [5, 13–15].

The second active measure was to ban admission of TB patients or patients suspected to have TB from the hospital hosting the transplant surgery department. As proved by the molecular survey, the current airborne prevention measures failed to protect three transplanted patients from TB infection. Additional measures to prevent any contact between lung transplant recipients and TB patients were therefore implemented. Since then, no other TB transmission in lung transplant recipients has been notified.

Finally, the third control measure adopted in response to the MDR-TB transmission event reported, was to accelerate the construction of an appropriate building equipped with 12 negative-pressure rooms, to improve TB control inside and outside the hospital.

In conclusion, *M. tuberculosis* genotyping and downstream real-time comparison of all isolates performed upon the diagnosis workflow allowed dissecting transmission events and optimising straightforward prevention measures. TB WGS-based genotyping coupled to contact tracing is a promising strategy to monitor TB dynamics of transmission in low prevalence area and high-income healthcare settings to promptly address control measures.

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References

1 World Health Organization. Global Tuberculosis Report. Geneva, WHO, 2018.
2 Lönnroth K, Migliori GB, Abubakar I, *et al.* Towards tuberculosis elimination: an action framework for low-incidence countries. *Eur Respir J* 2015; 45: 928–952.
3 Cabibbe AM, Trovato A, De Filippo MR, *et al.* Countrywide implementation of whole genome sequencing: an opportunity to improve tuberculosis management, surveillance and contact tracing in low incidence countries. *Eur Respir J* 2018; 51: 1800387.
4 Xu Y, Cancino-Muñoz I, Torres-Puente M, *et al.* High-resolution mapping of tuberculosis transmission: Whole genome sequencing and phylogenetic modelling of a cohort from Valencia Region, Spain. *PLoS Med* 2019; 16: e1002961.
5 Genestet C, Tatai C, Berland J-L, *et al.* Prospective whole-genome sequencing in tuberculosis outbreak investigation, France, 2017–2018. *Emerging Infect Dis* 2019; 25: 589–592.
6 Holden KL, Bradley CW, Curran ET, *et al.* Unmasking leading to a healthcare worker *Mycobacterium tuberculosis* transmission. *J Hosp Infect* 2018; 100: e226–e232.
7 Pichat C, Couvin D, Carret G, *et al.* Combined genotypic, phylogenetic, and epidemiologic analyses of *Mycobacterium tuberculosis* genetic diversity in the Rhône Alpes Region, France. *PLoS One* 2016; 11: e0153580.
8 Haut Conseil de la Santé Publique. Enquête Autour d'un Cas de Tuberculose. Recommandations Pratiques. [Investigation Around a Case of Tuberculosis. Practical Recommendations.] www.hcsp.fr/Explore.cgi/avisrapportsdomaine?clefr=391 Date last updated: 12 Nov 2013.
9 Abad CLR, Razonable RR. *Mycobacterium tuberculosis* after solid organ transplantation: a review of more than 2000 cases. *Clin Transplant* 2018; 32: e13259.
10 Lee RS, Proulx J-F, Menzies D, *et al.* Progression to tuberculosis disease increases with multiple exposures. *Eur Respir J* 2016; 48: 1682–1689.
11 Manabe YC, Dannenberg AM, Tyagi SK, *et al.* Different strains of *Mycobacterium tuberculosis* cause various spectrums of disease in the rabbit model of tuberculosis. *Infect Immun* 2003; 71: 6004–6011.
12 Yang C, Luo T, Sun G, *et al. Mycobacterium tuberculosis* Beijing strains favor transmission but not drug resistance in China. *Clin Infect Dis Off Publ Infect Dis Soc Am* 2012; 55: 1179–1187.
13 Nikolayevskyy V, Niemann S, Anthony R, *et al.* Role and value of whole genome sequencing in studying tuberculosis transmission. *Clin Microbiol Infect* 2019; 25: 1377–1382.
14 Arnold A, Witney AA, Vergnano S, *et al.* XDR-TB transmission in London: case management and contact tracing investigation assisted by early whole genome sequencing. *J Infect* 2016; 73: 210–218.
15 Walker TM, Merker M, Knoblauch AM, *et al.* A cluster of multidrug-resistant *Mycobacterium tuberculosis* among patients arriving in Europe from the Horn of Africa: a molecular epidemiological study. *Lancet Infect Dis* 2018; 18: 431–440.