Outbreak of pre- and extensively drug-resistant tuberculosis in northern Italy: urgency of cross-border, multidimensional, surveillance systems

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To the Editor:

Multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis (TB) are a major public health threat because of reduced treatment options and poor patient outcomes. New antimicrobials and regimens have been developed in recent years, but their effectiveness has been hampered by the rise and spread of drug-resistant strains. Whole genome sequencing (WGS) has emerged as a tool able to revolutionise TB surveillance and the clinical management of TB. This approach is now widely used by public health agencies in detecting outbreaks and transmission chains, thus supporting the formulation of evidence-based health policies. Moreover, WGS can predict the drug-susceptibility profile of *Mycobacterium tuberculosis* complex (MTBC) strains, often in combination with phenotypic antimicrobial susceptibility testing (AST) and minimum inhibitory concentration (MIC) determination for the optimal clinical management of drug-resistant TB [1].

In Italy (estimated MDR/rifampicin-resistant (RR) TB incidence of 0.3 per 100 000 population), like most Northern and Western European countries, the majority of TB cases, especially MDR/RR forms, are diagnosed in the foreign-born population, while the situation is reversed in Eastern European countries [2], where most of the cases affect the general population [3, 4].

Here, we describe a cluster of 16 sputum-smear positive pre-XDR- and XDR-TB (resistant both to fluoroquinolones and bedaquiline, therefore meeting the criteria for 2021 definition) [5] cases, involving individuals originally from different countries of the European Union (EU), that has been circulating in Northern Italy (figure 1a), specifically in the city of Milan and its hinterland between 2016 and 2020, and which represents a public health threat at EU-level if cross-border transmission is not adequately prevented.

All MTBC isolates underwent WGS as previously described [6]. Sequence data analysis (*i.e.* screening of variants, phylogenetic and relatedness analysis) was performed as described by Battaglia *et al.* [6] using the MTBseq pipeline (version 1.0.2) [7]. Isolates were considered as part of the cluster if sequencing data differed by six or fewer single nucleotide polymorphisms (SNPs). Phenotypic AST was performed on BACTEC MGIT 960 (Becton-Dickinson, Sunnyvale, CA, USA) according to the manufacturer's instructions and World Health Organization recommendations [8], while MIC testing was carried out on MGIT as described by Ghodousi *et al.* [9]. The epidemiological link was confirmed if cases were either direct contacts or had spent time in the same setting, or defined probable if cases, belonging to the same WGS-based cluster, were diagnosed and lived in the same city.

The index case (*i.e.* P01), a 29-year-old HIV-positive barman with no previous history for TB, was diagnosed with pre-XDR pulmonary TB, with additional borderline MIC for delamanid (MIC 0.06 $mg \cdot L^{-1}$) in October 2017, and reported being symptomatic for approximately 1 month before diagnosis. Since then, 15 TB cases were detected as part of a molecular cluster with P01 thanks to the routine use of WGS in the surveillance of MDR-TB (figure 1b). Notably, eight cases were also epidemiologically linked to P01 as they were either clients (*i.e.* P02 and P08) or workers (*i.e.* P03, P06, P07 and P10) of the nightclub where P01 worked, or friends (*i.e.* P04 and P05) hosted at home of one of

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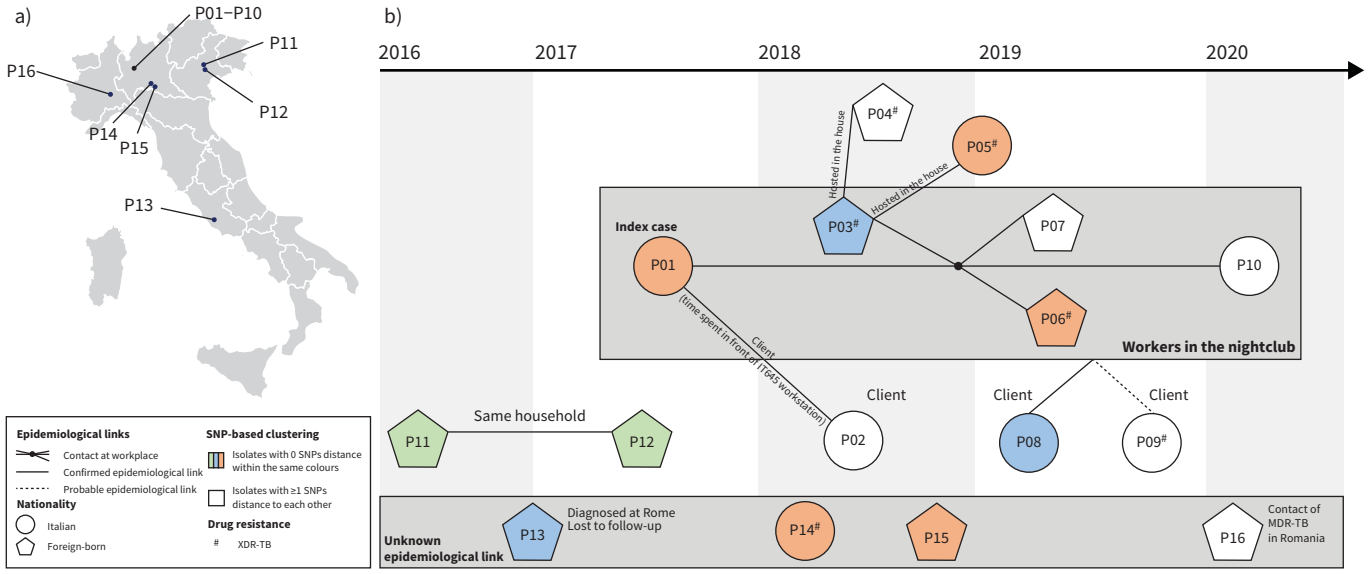

FIGURE 1 a) Spatial and b) temporal distribution and description of epidemiological links of clustered tuberculosis (TB) cases. MDR: multidrug resistant; XDR: extensively drug resistant; SNP: single nucleotide polymorphism.

them (*i.e.* P03). One patient (*i.e.* P09) denied having had contacts with other members of the epi-link cases or having spent time in the same setting.

The cluster included mostly males (11; 69%), young (median 26.5 years) and foreign-born (nine; 56%) individuals, mainly from Eastern Europe: five from Romania (*i.e.* P03, P06, P13, P15 and P16), two from Albania (P11 and P12) and one from Ukraine (*i.e.* P04). Notably, 12 of the 16 cases (*i.e.* P01–P07, P09, and P12–P15) were also identified as part of the cross-border cluster (*i.e.* snpCL1) recently described by Tagliani *et al.* [10]. Among the 16 clustered cases, six had XDR-TB (*i.e.* P03–P06, P09 and P14), eight pre-XDR-TB (*i.e.*, P01, P02, P07, P08, P10–P12 and P15), and two MDR-TB (*i.e.* P13 and P16) (figure 1b). Four cases (*i.e.* P05, P06, P14 and P15) had identical isolates to P01, based on SNP-based assessment, but only for two of them, P05 and P06, an epi-link to the index case was established. Three identical isolates, belonging to P03, P08 and P13, had one SNP difference from the index case isolate. Among those, only P13, a patient originally from Romania and diagnosed in Central Italy in 2016, was not epi-linked to P01. The remaining cases epi-linked to P01 had isolates differing by two or three SNPs from that of the index case. Among the not epi-linked cases, P16, a Romanian patient diagnosed in North-Western Italy in 2020, and whose isolate differed by five SNPs from that of P01, was identified as a close contact of an MDR-TB case in Romania, while P11 and P12, the two Albanian cases who belonged to the same household and were diagnosed in North-Eastern Italy in 2016–2017, had identical isolates four SNPs apart from that of the index case.

Apart from one patient (*i.e.* P03) previously treated for TB in 2013, no other patients had a prior history of TB, and were never exposed to anti-TB treatment, suggesting that resistance was likely the result of transmission rather than being acquired during treatment.

All isolates belonged to lineage 4.6. Six isolates (37.5%) were resistant to bedaquiline (*i.e.* P03–P06, P09 and P12), including one isolate also resistant to delamanid (*i.e.* P05) and one resistant to both delamanid and clofazimine (*i.e.* P04). Furthermore, three isolates had borderline MICs for delamanid (MIC 0.06 $mg \cdot L^{-1}$) (*i.e.* P01, P03 and P06); and three for clofazimine (MIC 1.0 $mg \cdot L^{-1}$) (*i.e.* P03, P05 and P06).

In this letter, we describe how the routine use of WGS-based surveillance of MDR/RR-TB allowed the identification of a mixed pre-XDR/XDR-TB cluster across Northern Italy, mainly among foreign-born individuals from Eastern Europe. This is the first XDR-TB transmission according to the new definition (*i.e.* resistance to two or more group A drugs) documented in the country. Although the exhaustive contact tracing activities allowed to establish epidemiological links between clustered cases, we were not able to confidently identify the source of the cluster that likely has cross-border ramifications in Northern Italy and Eastern Europe, as suggested by the overlap with cross-border cluster snpCL1 previously described [10].

Therefore, across the EU, public health experts must be aware that cross-border transmission of pre-XDR and XDR-TB could occur due to the European right to freedom of movement [11], and be unnoticed because of the lack of cross-border molecular surveillance integrated with health records and contact tracing data.

As already claimed [10], the implementation of a WGS-based surveillance system involving all EU member states is urgently needed to properly and promptly address such events and limit further transmission of highly resistant TB strains. This implementation may also serve and be transferred to the control of other infectious diseases. However, the benefits of establishing such coordinated cross-national surveillance might be attenuated and undermined if microbiological data are not coupled with patients' health records and contact tracing data, whose sharing is prevented by the current privacy legislation in data sharing between and within member states (*i.e.* article 20, General Data Protection Regulation). Hopefully, the extension of the mandate of the European Centre for Disease Prevention and Control, adopted in November 2020 by the European Commission [12], and the creation of a European Health Data Space, as proposed in September 2020 [13], may together enable the establishment of a more flexible and real-time epidemiological and molecular surveillance for infectious diseases, including for TB. However, these proposals are likely to fall short if highly mobile populations (*e.g.* seasonal workers, asylum seekers) [14] will be excluded in the design of such an ambitious integrated e-health platform.

In conclusion, the presence and the likely cross-border transmission of highly resistant TB strains needs to be addressed in a timely and effective manner. We urge for policymakers and public health experts to implement measures capable of preventing the cross- and within-border spread of extremely contagious and resistant pathogens.

Simone Villa [1,6], Elisa Tagliani [2,6], Emanuele Borroni[2], Paola Francesca Castellotti[3], Maurizio Ferrarese[3], Arash Ghodousi[2], Anna Lamberti[4], Sabrina Senatore[4], Marino Faccini[4,7], Daniela Maria Cirillo [2,7] and Luigi Ruffo Codecasa[5,7]

[1]Centre for Multidisciplinary Research in Health Science, University of Milan, Milan, Italy. [2]Emerging Bacterial Pathogens Unit, Division of Immunology, Transplantation and Infectious Diseases, IRCCS San Raffaele Scientific Institute, Milan, Italy. [3]Regional TB Reference Centre, Villa Marelli Institute and Laboratory/ASST Niguarda, Milan, Italy. [4]Health Protection Agency, Metropolitan Area of Milan, Milan, Italy. [5]Regional TB Reference Centre, Villa Marelli Institute, ASST Niguarda, Milan, Italy. [6]Co-first author (equally contributed). [7]Co-last author (equally contributed).

Corresponding author: Daniela Maria Cirillo (cirillo.daniela@hsr.it)

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