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Integrative transnational analysis to dissect tuberculosis transmission events along the migratory route from Africa to Europe

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Abstract

Background: Growing international migration has increased the complexity of tuberculosis transmission patterns. Italy's decision to close its borders in 2018 made of Spain the new European *porte entrée* for migration from the Horn of Africa (HA). In one of the first rescues of migrants from this region at the end of 2018, tuberculosis was diagnosed in eight subjects, mainly unaccompanied minors.

Methods: *Mycobacterium tuberculosis* isolates from these recently arrived migrants were analysed by Mycobacterial Interspersed Repetitive-Unit/Variable-Number of Tandem Repeat (MIRU-VNTR) and subsequent whole genome sequencing (WGS) analysis. Data were compared with those from collections from other European countries receiving migrants from the HA and a strain-specific PCR was applied for a fast searching of common strains. Infections in a cellular model were performed to assess strain virulence.

Results: MIRU-VNTR analysis allowed identifying an epidemiological cluster involving three of the eight cases from Somalia (0 single-nucleotide polymorphisms between isolates, HA cluster). Following detailed interviews revealed that two of these cases had shared the same migratory route in most of the trip and had spent a long time at

a detention camp in Libya. To confirm potential en route transmission for the three cases, we searched the same strain in collections from other European countries receiving migrants from the HA. MIRU-VNTR, WGS and a strain-specific PCR for the HA strain were applied. The same strain was identified in 12 cases from Eritrea diagnosed soon after their arrival in 2018 to the Netherlands, Belgium and Italy. Intracellular replication rate of the strain did not reveal abnormal virulence.

Conclusions: Our study suggests a potential en route transmission of a pan-susceptible strain, which caused at least 15 tuberculosis cases in Somalian and Eritrean migrants diagnosed in four different European countries.

Key words: Tuberculosis, trans-national, transmission, Horn of Africa

Introduction

Molecular epidemiology has significantly improved our understanding of the dynamics of tuberculosis (TB) transmission and allowed us to identify the hot-spots causing a higher number of secondary cases.[1–5] However, better analysis of clusters, splitting non-robust Mycobacterial Interspersed Repetitive-Unit/Variable-Number of Tandem Repeat (MIRU-VNTR) clusters, determining the direction of transmission, or even identifying super spreaders can only be done with Whole Genome Sequencing (WGS).[6–8]

The major social and epidemiological transformation experienced in many host countries receiving migrants has led to an increase in the complexity of TB transmission patterns. Recent transmissions after arrival must be clearly differentiated from endogenous reactivations, as each requires different management strategies from an epidemiological perspective. Some studies have demonstrated that MIRU-VNTR overestimates transmission in migrant clusters and only WGS can trace clustering of cases consistent with the epidemiological links.[9–11]

Besides the much-needed methodological refinements for the analysis of local TB transmission in settings with large number of migrants, the way TB etransmission is currently surveyed also requires changes in light of the new global TB scenario derived from increased migration. Molecular analysis of cross-border transmission by integrating isolates from migrants in multiple host countries with those prevalent at the countries of origin allows a more precise description of the current global dynamics of transmission.[9,12,13] A recent European Centre for Disease Prevention and Control (ECDC) pilot study used WGS integrative analysis to successfully identify the most relevant cross-border transmission events involving multi-drug resistant tuberculosis (MDR-TB) cases.[14]

Italy's decision to close its borders in 2018 moved Europe's front door to Spain for Sub-Saharan Africa migration route through Libya. Data from 2018[15] positioned Spain as the country with the highest number of entries (58 570, 46% of total arrivals versus 22 000 during the previous year), almost three-fold the figures in Italy for the same year 2018 (23 400, 17% of arrivals). The main African nationalities using the migratory route are Sudanese, Somalis, Moroccan, Ivorian and Eritrean,[16] previously not hosted by Spain.

To adapt to the analytical challenges of the new social and epidemiological complexity we used a triple approach: multi-national integrative analysis (collaborative effort to integrate the analysis of the isolates in four different European countries), WGS sequencing and strain-specific PCRs. This integrated strategy was essential to reveal an en route exposure event causing a trans-national outbreak in 2018, involving 16 cases. Most cases in this outbreak were unaccompanied minors from two different countries at the Horn of Africa (HA), Somalia and Eritrea, diagnosed with tuberculosis soon after their arrival to four different European countries: Spain, the Netherlands, Belgium and Italy.

Methods

Integrative analysis: databases and collections

Genotypic data of *Mycobacterium tuberculosis* (MTB) strains from four migrants from the HA, diagnosed with tuberculosis at arrival to Spain, were compared against the genotype of strains from the Netherlands, Belgium and Italy, three European countries also known to receive migrants from that African region.

An integrative analysis was performed, adapting the type of data available from each country. The following scheme was followed: (1) Comparison of MIRU-VNTR data from the isolates collected in Spain and the Netherlands; (2) WGS analyses of the three isolates sharing the HA-strain MIRU-VNTR pattern determined in the comparison carried out in step 1; (3) Design of a strain-specific PCR for the HA strain and its transfer to Belgium—where systematic MIRU-VNTR or genomic analysis is not carried out—to identify additional HA-strain representatives; (4) Confirmation by WGS that the isolates in Belgium identified by the strain-specific PCR corresponded to the HA-strain; (5) The HA-strain-specific PCR was not required in Italy as systematic WGS analysis is done. Thus, direct WGS comparisons were performed with the isolates collected in this country.

The following databases and collections were used to compare the HA strains identified in Spain: (i) in the Netherlands: the National Institute for Public Health and the Environment (*RIVM*), which includes 13 833 records, 12 708 of them of diagnostic samples, with a 24-loci VNTR profile of isolates from 1994 to (and including) 2019; (ii) in Belgium: collection of *M. tuberculosis* isolates from 80 (32 available) migrants who recently arrived from the HA, and were diagnosed during 2018; (iii) in Italy: collection of the clinical isolates available at the Regional Reference Centre for Advanced TB Diagnosis in the Calabria region and referred to Ospedale San Raffaele for WGS. This latter collection consisted of 48 randomly selected rifampin-susceptible strains available at the Reference Centre and collected

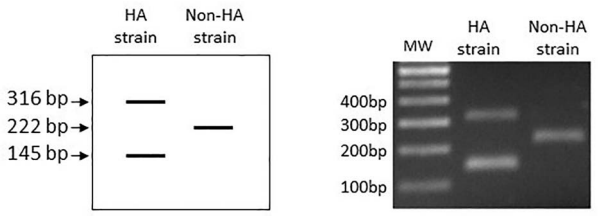

Figure 1. HA-strain-specific PCR: *In silico* ASO-PCR expected patterns for the HA strain and for any other non-HA strain (left panel) and real experimental patterns in an agarose gel (right panel). Length in base-pairs (bp) for each amplicon is indicated.

between 2013 and 2019, including 24 samples from TB patients arriving from Africa (five from the HA).

Mycobacterial Interspersed Repetitive-Unit/Variable-Number of Tandem Repeat analysis.

The 24-loci version of the internationally accepted MIRU-VNTR typing was applied,[17] modified as published elsewhere[18] (Details in Supplementary Methods).

The MIRU-VNTR patterns were used for comparing between Spanish and Dutch cases. For the comparison, Dutch cases were restricted to the 2016–2018 period, because it is the only period for which VNTR and WGS data were available for all cases. Systematic WGS started in 2016 and VNTR was discontinued in 2019.

Whole Genome Sequencing

WGS was performed as described elsewhere.[19] To determine relatedness between the isolates we followed the international consensus thresholds (<12 SNPs to consider probable relationship and <5 SNPs to determine certain relationships[7] (Details in Supplementary Methods).

Strain-specific ASO-PCR

Strain-specific SNPs were identified after comparing the SNPs extracted from WGS data against those from an in-house database (IBV-CSIC, Valencia, Spain), which contains the SNPs from 4761 strains and thus illustrates the geographic and phylogenetic diversity of the MTB complex.[20] The allele-specific-PCR (ASO-PCR) was designed following previously described methodologies.[21] Details in Supplementary Methods and Figure 1.

Infections in cellular model

U937 cell line monocytes were differentiated to macrophages and infected with the MTB variants. At Days 0 and 4 post-infection, cells were lysed and plated to determine CFUs. Ratios between CFU counts on Days 4 and 0 were calculated. Details in Supplementary Methods.

Epidemiological research

A detailed interview was conducted with two of the three Somalian cases diagnosed in Spain in the HA cluster. Data about the migratory trip were gathered: time of departure, countries crossed, time schedule for crossing each country, relationships between them along the journey, time in which they coincided along the trip and conditions of life at each country.

Results

In December 2018, 236 migrants (38% under 18 years of age) from 21 different sub-Saharan countries (52% from Somalia and Ivory Coast) were rescued from the sea in a single operation in front of the Libyan coast. After 1 week on board without permission to disembark from the Mediterranean countries closest to the Libyan coast, they were finally got off in Spain's Southern Coast. On arrival, eight TB cases, mostly unaccompanied minors, were diagnosed, seven from Somalia and one from Ivory Coast (Table 1). Four cases were smear microscopy-positive, two presented cavitated TB and another one disseminated TB (he died soon after).

MIRU-VNTR was performed directly on the sputum of three smear-positive cases. This allowed fast identification of a cluster involving two of the three cases; subsequent MIRU-VNTR from cultured isolates of the remaining cases confirmed the cluster, identified two additional clustered cases, and determined four orphan (unrelated) cases (Table 1).

We explored two alternative hypothesis for the cluster: (i) a common exposure along the migratory route or (ii) independent infections by a strain prevalent in their country of origin, Somalia.

To evaluate these two options, we searched for the same MIRU-VNTR pattern in the nation-wide MIRU-VNTR genotype database of the Netherlands, a regular receptor of migrants from the HA. Eight cases from the HA (mostly unaccompanied minors) diagnosed at an asylum seekers centre in the Netherlands shared the same MIRU-VNTR pattern of the four clustered cases from Somalia detected in Spain. All isolates were pan-susceptible, seven were cases from Eritrea and one from Somalia. Six cases were diagnosed 0–6 months after arrival in 2018, one 6–30 months after arrival in 2018, and the one 2.5–5 years following arrival in 2016.

The 12 isolates obtained in Spain and the Netherlands sharing the MIRU-VNTR pattern were analysed by WGS. A total of 10 revealed 0–1 SNPs genetic distance between them (0 SNPs: six cases among Eritreans in the Netherlands and three Somalian cases in Spain; 1 SNP: one Eritrean case in the Netherlands, Figure 2, Supplementary Table 1), indicating highly likely relationships between the cases. The other two isolates with the same MIRU-VNTR pattern (one of the cases diagnosed in Spain and the only Somalian case diagnosed in the Netherlands long time after arrival) did not cluster with the 0–1 SNP group (HA cluster), but were positioned in different branches of the network and accumulated higher diversity, 34 and 45 SNPs, respectively (Figure 2), indicating that they were unrelated with the 0–1 SNPs HA group.

The identity/high similarity determined by WGS analysis in most cases analysed in Spain and the Netherlands, who were linked to independent trips, and the fact that they corresponded to recently arrived cases from two different countries of origin, Somalia and Eritrea, favoured the hypothesis for the HA cluster:

Table 1. Patients' and strains' data for the cases in study from Spain

Pat	Ref	Country	MIRU-VNTR	Lineage	Cluster	WGS/SNPs	Gender	AGE	TB presentation	Smear result	MLVA	SNP_Barcode
1	SP2482	Somalia	225 133 253 645 324 244 247 423	L3 (Delhi/CAS)	Orphan	ND	F	17	Cavitated Pulmonar TB	3+	4539–32	3
2	SP2487	Somalia	225 132 254 313 243 242 323 323	L4 (Haarlem)	CLUSTER	0	M	17	Disseminated TB/Died	4+	673–69	4.8
3	SP2488	Somalia	225 132 254 313 243 242 323 323	L4 (Haarlem)	CLUSTER	45	M	17	Pulmonar TB	-	673–69	4.8
4	SP2491	Somalia	215 132 254 515 243 242 323 323	L4 (Haarlem)	Orphan	ND	M	18	Pulmonar TB	-	?-109	4.8
5	SP2494	Ivory Coast	215 132 253 333 244 252 535 423	L4 (Cameroon)	Orphan	ND	M	18	Cavitated Pulmonar TB	Pos*	11 698–26	4.6.2.2
6	SP2495	Somalia	225 132 254 313 243 242 323 323	L4 (Haarlem)	CLUSTER	0	F	16	Pleuro/pulmonar TB	2+	673–69	4.8
7	SP2501	Somalia	225 132 254 313 243 242 323 323	L4 (Haarlem)	CLUSTER	0	F	16	Pulmonar TB	-	673–69	4.8
8	SP2515	Somalia	223 132 253 533 233 433 637 221	L4 (Haarlem)	Orphan	ND	M	15	Pleuro/Pulmonar TB	-	291-?	4.6.2.1

*: the bacterial load was not specified
SP in the Ref code: Spain
MIRU-VNTR: Micobacterial-interspersed-repetitive-units Variable-number-tandem-repeats
WGS: Whole genome sequencing
SNPs: Single-nucleotide,polymorphisms
MLVA: Multiple loci VNTR analysis
SNP-barcode: Single-nucleotide-polymorphism phylogenetic barcode (F Coll code; NatureCommunications volume 5, Article number: 4812 (2014))

Figure 2. Genomic network for HA representatives. Each reference/code represents a patient's isolate. Each black dot represents an SNP (only SNPs in homozygosis are shown). Isolates within the same box share identical sequences (0 SNPs between them). The distribution of the isolates allows to identify the cases with likely relationships between them (0–1 SNPs between their isolates), from those unrelated (those located in different branches accumulating a higher number of SNPs between them. All cases were from Eritrea except those with the superscript +, who were from Somalia. *: In this isolate, insufficient coverage did not allow to confirm the presence of four of the 18 SNPs shared by the branch including the isolates sharing 0 SNPs. B, IT, N, SP: Isolates diagnosed in Belgium, Italy, the Netherlands and Spain. Numbers following the letters correspond to the reference-numbers given for the strains by the corresponding laboratories involved in the analysis in each country. REF: Genome used as a reference (most common ancestor for MTB, see Methods) to call SNPs. mv1: median vector, corresponding to not-sampled nodes.

an exposure hot-spot at some stage of the migratory route shared by migrants from different countries in the HA.

A detailed interview was conducted with two of the three Somalian cases diagnosed in Spain (HA cluster; 0 SNPs). They recognized having a long-term en route relationship. They had met at the beginning of the trip, when still crossing Somalia, and had travelled together until their arrival to Spain. This implies a prolonged epidemiological link: 1 month while crossing Yemen, 3 months in Sudan, and 18 months in Libya, where they stayed in the same detention camp (Figure 3).

Once the molecular identity and short genomic distances between the isolates were confirmed by epidemiological data, reinforcing our first hypothesis of en route transmission, we explored the possibility that the same strain could also be identified in other host countries in Europe, besides the Netherlands, that also receive migrants from the HA.

For tracking the HA strain in Belgium we designed a strain-specific PCR which was transferred to the Belgian National Reference Center (Supplementary Table 2). It was applied on the 36 MTB available isolates obtained from the 80 migrants from the HA diagnosed during 2018. An interpretable amplification pattern was obtained from 32 isolates (89%) and it allowed to identify three cases infected by the HA strain, all from Eritrea. Subsequent WGS analysis confirmed the three isolates to be the HA strain (0, 0 and 1 SNP deviation from the HA genomic cluster; Figure 2, Supplementary Table 1).

The application of the HA-strain-specific PCR was not required at the other surveillance node selected, Italy, as systematic WGS analysis was implemented. WGS comparative

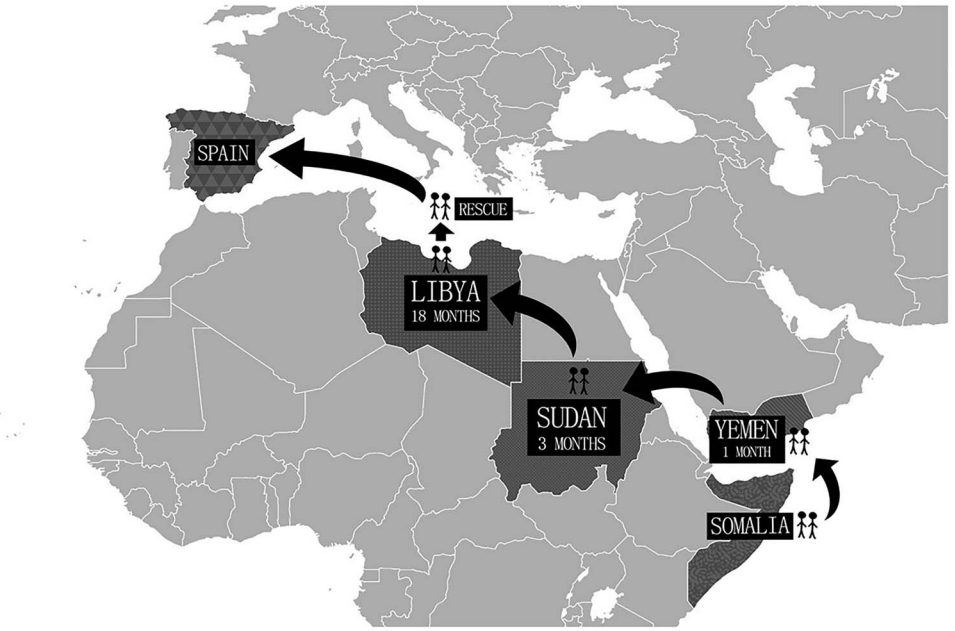

Figure 3. Migratory route. The journey followed by the two interviewed cases is indicated, specifying the countries they passed through and the time spent at each country. Map taken from https://freevectormaps.com/world-maps/WRLD-EPS-01-0016?ref=atr.

analysis allowed us to identify two new pan-susceptible cases differing in 0 SNPs from the HA group (Figure 2, Supplementary Table 1). The patients came from Eritrea and were diagnosed at arrival to two distinct Emergency Reception Centres of the Region during 2018 and then referred to the infection disease wards of the Hospitals in Crotone and Vibo Valentia, two cities of the Region, which likely ruled out their coincidence at same Centre. The collection from Italy was integrated with the WGS data available from other Italian Regions (around 1800 isolates including RS and RR/MDR isolated between 2006 and 2020, with around 30 isolates coming from the HA), however not identifying any additional coincidence with the HA group.

The results from four European countries, with MTB isolates corresponding to the HA cluster, suggested common exposure during the travel of subjects coming from different countries who followed the same migratory route. However, we were also interested in evaluating whether an increased infectivity of the strain may have had an influence on the transmission event, which would explain the large number of cases after exposure. Thus, U937 cells were infected (Supplementary Methods) with two representatives of the HA strain. Controls were an isolate from the HA sharing the same MIRU-VNTR pattern but not closely related (44 SNPs; SP-2488, Figure 2) and an isolate from Somalian migrants diagnosed at arrival into Spain but with a different MIRU-VNTR pattern (SP-2515, Table 1). The ratio for the CFU count between Days 4 and 0 was equivalent for the two HA isolates, consistent with their genomic identity, although they did not show higher growth efficiency within macrophages. Moreover, one of the two control strains rendered higher intra-macrophage growth values (Figure 4). A parallel assay using three of the isolates from the Netherlands (0 SNPs in comparison to those isolated in Spain) was performed. Similar results (not higher macrophage replication compared to the controls) were obtained (data not shown).

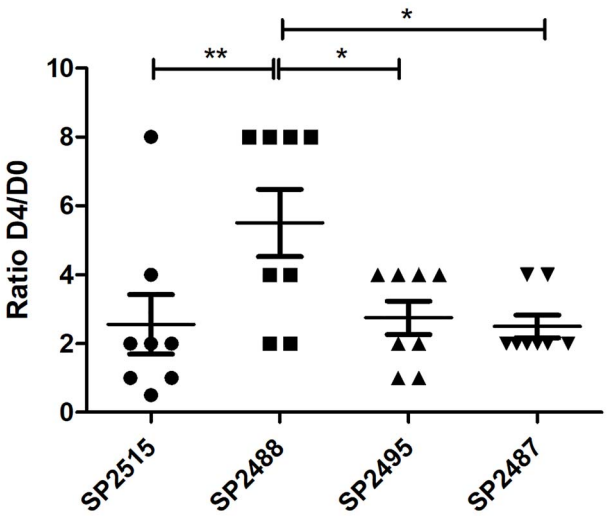

Figure 4. Intra-macrophage division rates. Ratios for CFU counts between Days 0 and 4 post-infection for two representatives of the HA strain (SP2495 and SP2487) and two controls (one from Somalia differing in 26 SNPs, SP2488 and a MIRU-VNTR-defined orphan strain form Somalia, SP2515) strains.

Discussion

The epidemiological scenario of TB has dramatically changed in many European countries due to international migration. Moreover, this new socio-epidemiological complexity is constantly fluctuating depending on the changes of migratory routes.

A change in the migratory behaviour has recently occurred in Spain. The traditional entry to Europe through Italy of migrants from the HA was modified due to a decision of Italy to close its borders in 2018, which positioned Spain as the new door of entrance. One massive rescue of migrants coming from the HA,

among whom eight subjects were diagnosed with TB, justified this study.

Despite an initial small sample size, the following was observed: (i) five cases were representative for the import of different strains, (ii) three cases were infected by the same HA strain and (iii) one case harboured a related variant of the HA strain, but with greater SNP-based diversity.

The most obvious explanation for the clustered cases was on-board exposure. However, 1 week is rather a short time to justify exposure and development of the disease. In addition, the clinical findings were more consistent with advanced disease, ruling out recent contagion. In fact, in two cases stain results were positive and one had disseminated TB and died from TB soon after. We considered an alternative explanation for the clustered cases, namely that the affected subjects imported a strain prevalent in their country of origin, Somalia.

The most direct way to assess the importation hypothesis would have been to compare the HA strain pattern with those from MTB strains circulating at the countries of origin at the HA. Unfortunately we did not have access to databases including these data. Alternatively we adopted a transnational analytical approach to analyse the isolates that had migrated from the HA and arrived to other European countries. We found 12 additional cases in the Netherlands, Belgium and Italy, who had come during 2018 from Eritrea, whose isolates shared the same HA strain with the three cases from Somalia, who had been diagnosed in Spain.

The fact that migrants from two different countries, Somalia and Eritrea, shared the same HA strain, with extremely low inter-isolate diversity (13 isolates with identical sequences, with 0 SNPs between them, and two isolates differing in only one SNP), made the hypothesis of a founder effect—a common strain at origin—an unlikely possibility and led us to consider the hypothesis of an en route recent transmission.

En route transmission had already been suggested for the extensive 1064-32 cluster in the Netherlands.[10] More recently, this was strongly demonstrated in a study by Walker *et al*.[22] who integrated cross-border analysis of MTB isolates, WGS and refined epidemiological interviews, to describe a MDR-TB outbreak involving 29 cases mostly from Sudan and the HA in seven European countries.

Our study constitutes the second detailed description of an *en route* transmission event having wide cross-border impact. It includes 15 cases coming from the HA distributed in four European host countries. To identify spatio-temporal coincidences along the migration route requires a thorough interview with the affected subjects, implying trust and collaboration when describing their difficult journey. We succeeded in performing these interviews in two of the three cases diagnosed in Spain, from which it was possible to identify several exposure opportunities as they shared most of the trip. The longest time spent together was in Libya in a detention camp at which they stayed for several months. Interviews were not possible with subjects detected in Italy, Belgium and the Netherlands. However, it is possible they also remained in a detention camp in Libya for a long time. Libya has been shown to be a hot-spot of exposure, specifically one detention camp, by Walker *et al*.[22] in the MDR outbreak study with cases coming from the HA.

WGS-based cross-border surveillance for systematically tracking the most relevant transnational MDR-TB transmission events in Europe has proved to be efficient and feasible.[14] This ECDC pilot study identified the three major cross-border clusters and included 30, 16 and 12 cases diagnosed in three, four and four European countries, respectively. These figures indicate that the magnitude of the event described in our study for a pan-susceptible MTB strain is similar to the biggest events involving MDR-TB identified by the European network of TB Reference Centers.

As shown in the ECDC study and our analysis, WGS resolution ensures the discriminatory power required for understanding the new complexity of migratory TB transmission. Standard molecular epidemiology, based on MIRU-VNTR analysis, has caveats when it comes to this point. We applied WGS on MIRU-VNTR-defined clusters from Almeria (rich in migrants from a single country) and detected two overlapping situations that engaged our attention: related cases, most likely due to recent transmission, and unrelated cases, due to the import of independent similar variants. Another study in Switzerland also found that MIRU-VNTR-based molecular epidemiology overestimates the rate of transmission among migrants, as WGS preferentially splits MIRU-VNTR migrant clusters, over autochthonous clusters.[11] An extended cross-border cluster of cases mainly from the HA in the Netherlands and Denmark was almost entirely split by WGS.[10]

Despite being WGS key for accurate global surveillance of migration-related transmission, systematic WGS is only performed in few settings. The use of strain-specific PCRs, targeting the strains to be surveyed, may help expand the surveillance beyond the network of Reference Centers. It may also allow to integrate within the same surveillance program not only European nodes, but also the laboratories at the countries of origin, most likely able to run standard PCRs. It was only when the host countries and the countries of origin were included that full understanding of intercontinental transmission events of MDR-TB between Peru and Europe was possible[12,23] by locally applying strain-specific PCRs at origin. In this current study, a strain-specific PCR allowed the rapid identification of the three cases in Belgium. This may be applied from now on, at minimal cost, to update the presence of the HA strain in any country.

Finally, our data seem to indicate that an epidemiological factor per se—spatio-temporal coincidence at Lybia or elsewhere along the migratory trip—may be enough to explain the en route transmission described in this study. However, the large number of cases linked to different trips throughout 2018 was unexpected. It suggests a potential additional role for the strain itself. The combination of a high-burden exposure hot-spot, and a highly fit strain, with a higher ability to be transmitted and/or infect secondary cases, may explain the large number of TB cases involved. In our case, the HA strain did not show higher intra-macrophage replication rates in comparison to two different strains identified in other Somalian cases in the same arrival event. We must acknowledge that we limit our observations to the ability of the strain to replicate in a cellular model without addressing any putative immunomodulatory effects, and additional work in this direction should be performed. Strains involved in other outbreaks of big magnitude, such as the Beijing

strain responsible for a large percentage of the cases in Grand Canary island (GC strain),[24] also behaved as average strains as per the rate of multiplication in macrophages.[25] This seems contradictory with the fact that the GC Beijing strain has remain present at high rates over the last decades and has also migrated to other neighbouring islands.[26] Nevertheless, it also illustrates how epidemiological factors by themselves may be sufficient for high-magnitude transmission events. For example, in Grand Canary island, probably the high burden of exposure caused by an index case with high bacillary load and a prolonged diagnostic delay[27] and in our study, high burden exposure hotspot throughout the migratory route.

Our data support the need of considering en route transmission before the arrival to the host countries in the analysis of migration-related clusters. The traditional dichotomous approach of labelling a new TB migrant case as either recent transmission at the host country, when clustered with other cases, or to a reactivation due to an exposure at origin, when it corresponds to an orphan genotype is rather naive in the current context of epidemiological complexity. Accurate identification of en route transmission cases will rule out misinterpretations or interferences in the identification of active transmission chains after the arrival of migrants. It will allow properly targeting the surveillance resources providing maximum efficiency and control. Multinational integrative efforts must be activated not only for MDR TB outbreaks but also for pan-susceptible strains, if we aim a further more extensive understanding and efficient control of global TB transmission. Integration of molecular/genomic datasets with new information provided by simple strain-specific PCRs was essential to correctly interpret the factors responsible for the events we have described in this study.

Supplementary data

Supplementary data are available at *JTM* online.

Author Contributions

MML: Research, funding, epidemiological analysis, conceptualization.

RJ: Research, MS revision.

VM: Research, MS revision.

AM: Research, MS revision.

AMC: Research, MS revision.

AV: Research, MS Revision.

PSC: Bioinfromatics.

EA: Research.

SRM: Technical analysis.

JAGC: Sequencing and genotyping.

MB: Epidemiological research.

ACO: Bioinformatics.

BL: Epidemiological research.

SVG: Epidemiological research.

IC: Conceptualization, MS Revision.

PM: MS Revision, Funding.

DMC: MS Revision, Funding, Conceptualization.

DVS: Conceptualization, MS Revision.

LPL: Research, MS Revision, Design, analysis, conceptualization.

DGV: Design, Research, MS writting, analysis, conceptualization, funding.

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