Exportation of MDR TB to Europe from Setting with Actively Transmitted Persistent Strains in Peru

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We performed a cross-border molecular epidemiology analysis of multidrug-resistant tuberculosis in Peru, Spain, and Italy. This analysis revealed frequent transmission in Peru and exportation of a strain that recreated similar levels of transmission in Europe during 2007–2017. Transnational efforts are needed to control transmission of multidrug-resistant tuberculosis globally.

International migratory movements have created a need for cross-border surveillance of tuberculosis (TB). Monitoring the transmission of multidrug-resistant (MDR) *Mycobacterium tuberculosis* strains deserves further analysis (*1*). Through migration, MDR strains can become more widely dispersed; they can be exported from the 30 countries with 89.7% of the incident MDR cases (*2*) to lower prevalence settings.

We performed a transnational molecular epidemiology analysis of MDR TB cases covering a setting with one of the highest resistance rates in Latin America (Lima, Peru) (*2*) and 2 settings in Europe hosting immigrants from Peru (Florence, Italy; and Madrid, Spain) to identify incidents of cross-border transmission. We selected 60 consecutive MDR TB cases (20% of the total MDR cases in Lima) diagnosed during 2014–2015 in one of the poorest districts of Lima (San Juan de Lurigancho), which has the highest incidence of TB (193 cases/100,000 population) in Peru (*3*). MIRU-VNTR (mycobacterial interspersed repetitive unit–variable-number tandem-repeat) analysis (Appendix 1, https://wwwnc.cdc.gov/EID/article/25/3/18-0574-App1.pdf) suggested a high percentage of recent transmission that included 36 (60%) of 60 isolates in 9 clusters (Appendix 2, https://wwwnc.cdc.gov/EID/article/25/3/18-0574-App2.xlsx). A comparison of these isolates with 228 genotyped isolates from the same district 4 years earlier (*3*) revealed that 6 of the 9 strains actively transmitted during 2014–2015 were present in 2011 (Appendix 1 Table 1).

We then investigated whether some of these persistent MDR TB strains actively transmitted in Lima could have been exported to Europe. We used a dataset of 87 MIRU-VNTR genotypes of isolates in Florence obtained from TB cases in Peru during 2001–2010 (*4*) and >300 MDR genotyped isolates obtained nationwide from Italy (*5*). We found that 1 genotype matched between the Lima and Italy MDR datasets; this genotype corresponded to a strain (C8-LPMDR) that infected 11 persons in Florence and 2 in Milan during 2007–2017 (Appendix 1 Table 2). MDR TB strains from Lima were also found in Spain during 2003–2009. Three MDR isolates, matching 3 of the 9 MDR TB strains from Lima, were found in migrants from Peru residing in Madrid (Appendix 1 Table 1). One of these isolates corresponded to the active MDR strain circulating in Italy (C8-LPMDR).

We performed whole-genome sequencing (*6*) with 12 of the 17 isolates of the cross-border MDR TB cluster C8-LPMDR (7 from Florence, 2 from Milan, 2 from Lima, and 1 from Madrid). In a median-joining network analysis, these isolates were distributed along 2 branches (Figure). One branch included all the isolates from Florence. Although we lacked precise data from contact tracing to verify details regarding transmission in Florence, we were able to determine that all the Peru migrants involved came from Lima. In Florence, there is a large community of persons from Peru, which offers opportunities for interacting, such as shared residence and social gatherings. The few differences (0–2 single-nucleotide polymorphisms [SNPs]) found among these isolates strongly suggests these isolates were recently transmitted in Florence. An isolate from Lima (6068) was only 3 SNPs different from a Florence isolate, demonstrating a close genetic

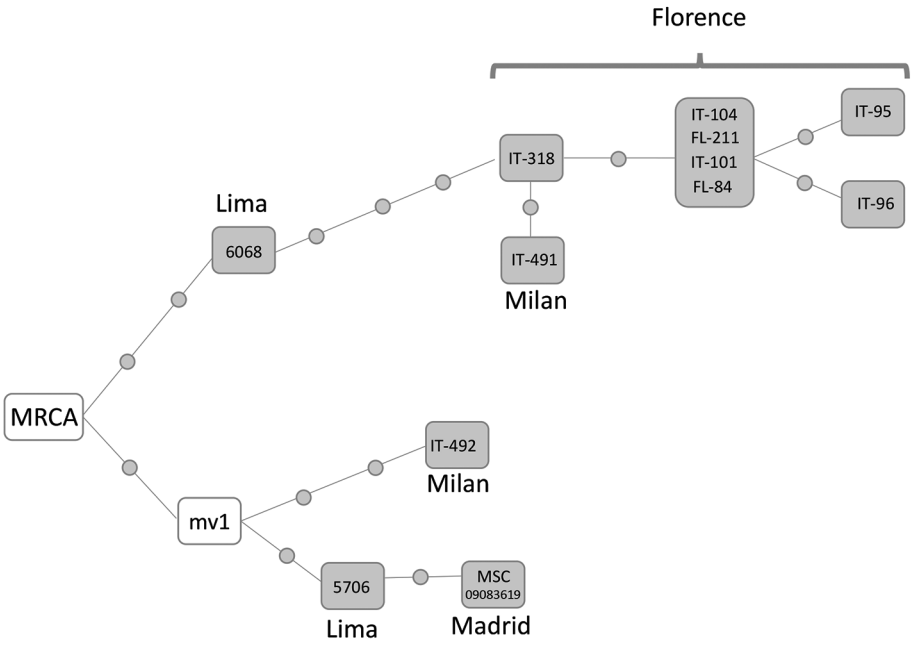

Figure. Median-joining network of whole-genome sequenced isolates of strain C8-LPMDR found in Italy, Peru, and Spain, 2007–2017. Network 4.6.1.6 (http://www.fluxus-engineering.com) was used to perform network analysis. Each dot along the lines linking isolates corresponds to a single-nucleotide polymorphism difference. Isolates within the same box share identical sequences. mv1 corresponds to an unsampled case inferred from the network topology. Sequences were deposited in the European Bioinformatics Institute database (http://www.ebi.ac.uk, accession no. PRJEB25765). FL, Florence; IT, Italy; MRCA, most recent common ancestor.

relationship between the Florence and Lima isolates. This close relationship also suggests that the starting point of this branch was an exportation event of an isolate from Lima. The second branch in the network includes 2 isolates identified in Europe (1 Madrid [city of origin unknown, data not available] and 1 Milan [origin Lima]) and 1 isolate identified in Lima. Because the most recent common ancestor is positioned between the 2 branches and the 2 isolates from Lima are in different branches, these branches probably represent 2 independent exportations of 2 variants of a strain prevalent in Lima that diversified after a prolonged period.

These data reveal that high-risk strains are being exported from Lima to 2 countries of Europe (Italy and Spain). Not only were these strains exported from Lima, but 1 strain caused a prolonged and ongoing transmission event in Italy. The transmission of this strain has caused at least 3 cases in Lima, 11 in Florence, 2 in Milan, and 1 in Madrid.

In another report, the international distribution of an MDR TB strain that caused 10 cases across 3 countries of Europe (Romania, Austria, and Germany) was investigated (*7*). The exportation event discussed in our report is geographically wider (intercontinental, from South America to Europe), involved more cases (17 total, with a transmission cluster of 12 cases in Italy), and occurred over a more extended period (secondary cases spanned 11 years).

Only integrative transnational efforts can provide a clearer picture of transmission of MDR TB, which has become more complex because of international migration. In this cooperative analysis involving Peru, Italy, and Spain, we detected a serious problem of active MDR TB transmission in Lima. This situation led to a pool of persistent strains that were responsible for similar transmission events after exportation to Europe via migration.

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Rectal Lymphogranuloma Venereum, Buenos Aires, Argentina

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Among 34 men with proctitis in Buenos Aires, Argentina, 16 (47%) had *Chlamydia trachomatis* infection, 11 (68.8%) of which were biovar lymphogranuloma venereum. The outbreak was probably local, as in Europe. In Argentina, lymphogranuloma venereum should be a suspected cause of proctitis in HIV-infected men who have had unprotected anal sex with men.

Lymphogranuloma venereum (LGV) is a sexually transmitted infection caused by *Chlamydia trachomatis* serovars L1, L2, or L3 and their variants. LGV has been considered endemic to Asia, Africa, and the tropical region of South America. Over the past 2 decades, case reports of LGV in Argentina have been sporadic and regarding only patients who acquired the infection abroad.

In the Netherlands in 2003, an outbreak of rectal LGV among men who have sex with men (MSM), mainly HIV infected, was reported (*1*). This report was followed by many other reports from other developed countries (*2*,*3*).

LGV has been traditionally described as causing inflammation and swelling of the inguinal lymph nodes and also involving the rectum, causing acute proctitis, particularly among HIV-infected MSM (*4*). Since 2015, some clinicians in Argentina have suspected LGV in certain patients with proctitis (regardless of association with inflammatory tumors) in which *C. trachomatis* has been detected but not genotyped. Thus, we conducted a prospective study to assess the *C. trachomatis* genotypes as the causative agent of infectious proctitis in Buenos Aires, Argentina. Our study was conducted in a private practice and a public hospital, under a protocol previously approved by the hospital's ethics committee (no. 201723).

From September 1, 2017, through February 1, 2018, we included in our study every man who visited either the private or public study site and who had rectal signs or symptoms of proctitis and had not taken antimicrobial drugs in the previous month. None of the included patients was referred by a previously included patient. Each participant signed an informed consent form.

Over the first 5 months, we obtained a rectal swab sample from 34 men on their first visit. To detect *C. trachomatis*, we extracted DNA from the samples by using real-time PCR targeting a cryptic plasmid fragment (Alert PCR; ELITech Molecular Diagnostics, https://www.elitechgroup.com). Positive samples were genotyped by *ompA*-based PCR restricted fragment length polymorphism (*5*).

Of the 34 samples analyzed, 16 were positive for *C. trachomatis*; 11 were identified as genotype L2 and 5 as genotypes D, F, or J. All participants reported having engaged in unprotected receptive anal sex in Argentina, except for 1 who had had receptive anal sex while in Mexico. None declared having traveled to an LGV-endemic area. Mean age was 31.63 years (range 22–43 years). All

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Appendix 1

Actively Transmitted Multidrug-Resistant Tuberculosis Isolates in Lima, Peru

The 60 multidrug-resistant (MDR) *Mycobacterium tuberculosis* isolates from consecutively diagnosed cases occurring during 2014–2015 in San Juan de Lurigancho (district of Lima, Peru) included in this study were assigned to lineages on the basis of lineage marker single-nucleotide polymorphisms (SNPs) and/or spoligotype (*1*,*2*). Of the 60 isolates, 56 corresponded to lineage 4 (L4) and the remaining 4 to lineage 2 (L2). MIRU-VNTR (mycobacterial interspersed repetitive unit–variable-number tandem-repeat) analysis (*3*,*4*) revealed that the most frequent sublineages were Latin America–Mediterranean (L4.3, 65%), Haarlem (L4.1.2, 15%), and Beijing (L2, 6.7%) (Appendix 1 Table 3).

MIRU-VNTR–based fingerprinting indicated that 36 (60%) of 60 isolates were included in 9 clusters (C1-LPMDR to C9-LPMDR; 2–10 isolates/cluster; Appendix 2, https://wwwnc.cdc.gov/EID/article/25/3/18-0574-App2.xlsx), suggesting a high percentage of recent transmission. One of the clusters with the fewest numbers of isolates (N = 2, C9-LPMDR) was of the Beijing lineage. Each cluster included cases distributed among various health centers. The percentage of clustered cases in our analysis was higher than average values obtained elsewhere, even for susceptible isolates.

Our sample included only 20% of the total MDR isolates in Lima, and the recruitment period we used was shorter than that recommended (*5*) to efficiently capture transmission clusters. Therefore, the true proportion of transmission could be even higher. MDR *M. tuberculosis* transmission in Lima is multifactorial; in an investigation involving whole-genome sequencing and social network analysis (*6*), exposure to healthcare venues, schools, and transportation were associated with MDR *M. tuberculosis* infection.

Persistent MDR *M. tuberculosis* Strains Actively Transmitted in Lima

Once highly active transmission of MDR *M. tuberculosis* in Lima was identified, we moved on to evaluate whether the MDR *M. tuberculosis* strains that were being transmitted constituted a persistent problem in Lima. To clarify this issue, we took advantage of genotyping data available for 228 isolates (including MDR, monoresistant, polyresistant, and susceptible isolates) from new tuberculosis cases in the same district of Lima 4 years earlier (during March 2010–December 2011). The integration of both data sets revealed that 6 of the 9 clustered strains actively transmitted during 2014–2015 were previously found in 2011 (Appendix 1 Table 1). Four clusters (the 2 with the largest number of isolates [C1-LPMDR and C2-LPMDR] and 2 with few isolates [C4-LPMDR and C9-LPMDR]) of the 6 were already MDR, and 3 of these 4 strains were already part of MDR transmission clusters in 2011 (2 cases C1-LPMDR, 4 cases C9-LPMDR, and 6 cases C2-LPMDR).

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Appendix 1 Table 1. Isolates sharing MIRU-VNTR type with multidrug-resistant tuberculosis clusters from Lima, Peru, 2014–2015*

Cluster	No. isolates in Lima, Peru, 2014–2015	No. isolates in Lima, Peru, 2010–2011	No. isolates in Florence, Italy, 2007–2017	No. isolates in Madrid, Spain, 2003–2009	Total no. isolates
C1-LPMDR	10	3†			13
C2-LPMDR	8	20‡	2§		30
C3-LPMDR	4				4
C4-LPMDR	3	1			4
C5-LPMDR	3	1†			4
C6-LPMDR	2			1	3
C7-LPMDR	2			1	3
C8-LPMDR	2	1†	13¶	1	17
C9-LPMDR	2	4			6
Total	36				

*All numbers without a footnote symbol correspond to multidrug-resistant isolates. MIRU-VNTR, mycobacterial interspersed repetitive unit–variable-number tandem-repeat.
†One monoresistant isolate.
‡Two polyresistant, 5 monoresistant, and 7 susceptible isolates.
§One polyresistant and 1 monoresistant isolate. Any of the isolates were multidrug resistant.
¶Two of these isolates were obtained from cases in Milan, Italy.

Appendix 1 Table 2. Characteristics of isolates of cluster C8-LPMDR, Italy, 2007–2017

No.	City of isolation	Location of origin	Year of isolation
1	Florence	Latin America	2007
2	Milan	Peru	2009
3	Florence	Peru	2010
4	Milan	Latin America	2011
5	Florence	Latin America	2012
6	Florence	Peru	2013
7	Florence	Italia	2013
8	Florence	Albania	2013
9	Florence	Peru	2013
10	Florence	Latin America	2014
11	Florence	Latin America	2014
12	Florence	Peru	2016
13	Florence	Peru	2017

Appendix 1 Table 3. Multidrug-resistant isolates from Lima, Peru, 2014–2015, by lineage

Lineage	No. isolates (%)
Latin America–Mediterranean	39 (65)
Haarlem	9 (15)
Cameroon	3 (5)
X	3 (5)
S	2 (3.3)
Beijing	4 (6.7)
Total	60