



## SURVEILLANCE REPORT

# Molecular typing for surveillance of multidrug-resistant tuberculosis in the EU/EEA

March 2017

## Summary

This report describes the geographical and temporal distribution of multidrug-resistant (MDR) tuberculosis (TB) using molecular typing data reported by European Union (EU)/European Economic Area (EEA) Member States for 2015 and the preceding years.

Overall, 22 EU/EEA Member States reported data covering 2 869 MDR TB isolates with complete mycobacterial interspersed repetitive units (MIRU) - variable number of tandem repeats (VNTR) 24 loci for the years 2003 to 2015 to the European Surveillance System (TESSy). In 2015, 18 Member States participated in the scheme for reporting molecular typing data, including Slovenia which had no notified cases of MDR TB. The overall typing coverage among all MDR TB strains in the 17 participating Member States reporting the notification of at least one case was 90.4%. Of 376 MDR TB strains isolated in 2015, 152 (40.4%) belonged to one of the 44 cross-border molecular clusters of MDR TB strains isolated in 2012–2015. The cluster size varied from two to 170 strains, involving two to 16 Member States. The two most common strains were 100-32 and 94-32, accounting for 30.7% and 16.8% of the clustered isolates, respectively. A lineage was assigned for 55.8% of the MDR TB strains isolated in 2015. More than half of the strains with assigned lineage (60.0%) belonged to the Beijing genotype, followed by LAM, TUR, and Haarlem, representing 17.6%, 9.0% and 5.2% of all strains, respectively.

The typing coverage at EU/EEA level and representativeness of typing data remain sub-optimal. Of the five EU/EEA high-priority MDR TB countries (Bulgaria, Estonia, Latvia, Lithuania and Romania), only Bulgaria and Estonia reported data for 2015. Despite these limitations, EU/EEA level analysis of molecular typing data facilitates the identification of strains that might be involved in cross-border transmission of MDR TB, and therefore helps to control the further spread of MDR TB.

## Background

The mycobacterial interspersed repetitive units (MIRU) - variable number of tandem repeats (VNTR) 24 loci method has become a standard tool for molecular typing of *Mycobacterium tuberculosis* strains in tuberculosis (TB) control programmes in many EU/EEA Member States.

From 2009 to 2012, the European Centre for Disease Prevention and Control (ECDC) funded a project on molecular typing for surveillance, to assess the risk of international transmission of multidrug-resistant (MDR) TB and extensively drug-resistant (XDR) TB in the European Union (EU)/European Economic Area (EEA). This project was outsourced to the TB Reference Laboratory at the National Institute for Public Health and the Environment, Bilthoven, the Netherlands.

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Erratum: on 14 July 2017 two lines of data in Table 1 were swapped for Norway and Portugal as these had been displayed in the incorrect order.

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In January 2013, ECDC initiated a pilot study to gain first-hand experience in the collection and analysis of molecular typing data at EU/EEA level and to assess the potential benefits, challenges and resource needs. The study was limited in scope (*Salmonella*, *Listeria*, Shiga toxin-/verocytotoxin-producing *E. coli* and MDR TB) and time (January 2013 to May 2014). The pilot evaluation revealed a need to improve the functioning, flexibility and user-friendliness of the tools for data submission and cluster visualisation and analysis. Although the assessment of the public health usefulness of EU-level molecular typing for surveillance was limited by the timeframe of the pilot, the participants recognised the relevance of molecular typing for surveillance of MDR TB at the EU level. The main recommendation for MDR TB molecular typing for surveillance was not to continue collecting data on a real-time basis, as the large reporting delay and low typing coverage in some Member States did not justify monthly cluster analyses. As an alternative, the submission of MDR TB isolate data was to be integrated into the annual TB data reporting to TESSy, so that both isolate- and case-based data could be submitted within the same time-frame.

A network expert group was established to support ECDC in analysing the data annually and producing a status report on the dynamics, geographic distribution and trends of MDR TB strains and clusters in Europe.

This second annual report focuses on strains isolated in 2015.

## Overview

### Data

Data pertaining to MDR TB strains isolated from 2003 to 2015 were extracted from TESSy on 31 October 2016. During this period, 2 869 MDR TB isolates with complete MIRU-VNTR 24 loci data were reported by 22 countries (Table 1). In 2015, data were reported for 376 MDR TB strains by 17 Member States, and one country reported zero MDR TB cases notified (Slovenia). Altogether, typing data were reported for 26.2% of 1 435 MDR TB cases notified in the EU/EEA in 2015. The typing coverage of MDR TB cases is lower than the updated data for 2014 (28.4% of 1 463) [1]. In 2016, Portugal reported molecular typing data to TESSy for the first time, providing data for strains isolated in 2014 and 2015. Germany did not report any molecular typing data for strains isolated in 2015.

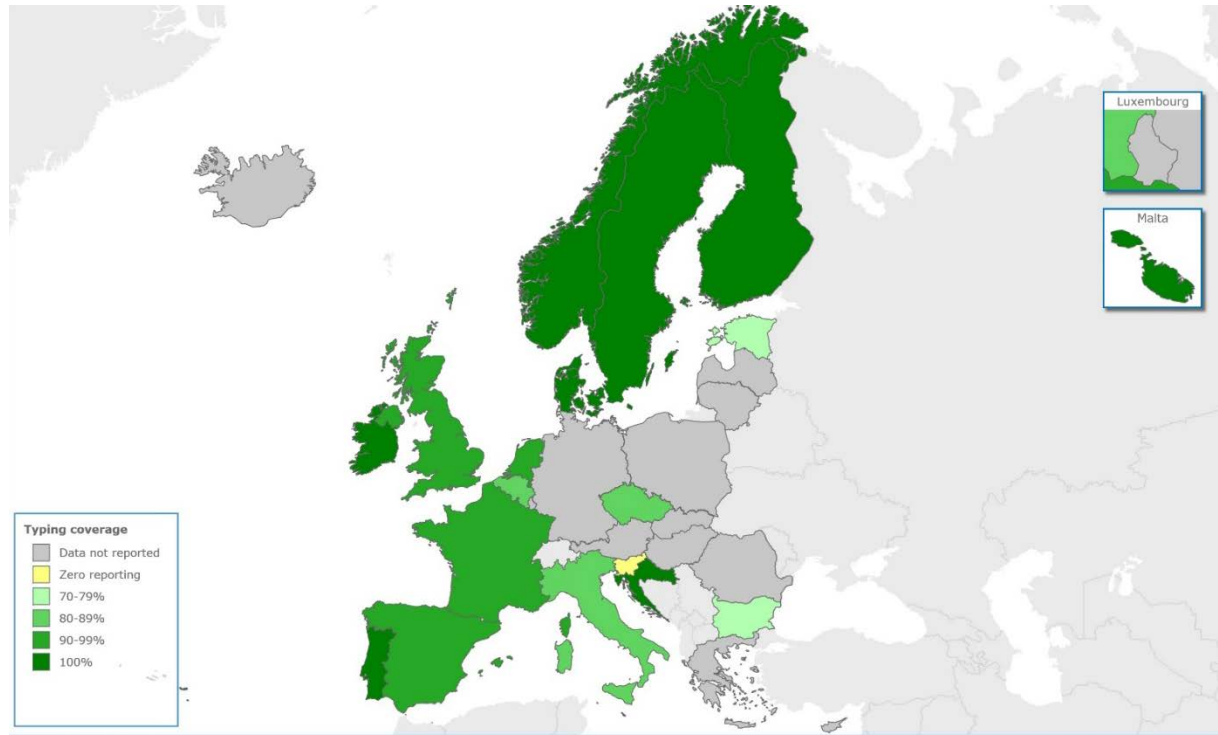
**Table 1. Number of MDR TB strains typed by MIRU-VNTR (24 loci) and reported to TESSy, EU/EEA, 2003–2015**

Country	Year													Total
	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	
Belgium							10	20	14	22	12	13	13	104
Bulgaria					20	20	38	42	45	34	18	35	17	269
Croatia	15	5	7	11	3	3	6	3	2	2		2	1	61
Cyprus						1								1
Czech Republic	6	2	6	5	1	9	6			4		5	8	52
Denmark	0	0	5	3	2	2	2	2	3	1	2	2	6	30
Estonia	101	79	84	58	78	66	74	54	58	53	31	24	28	788
Finland	3		2	2	2	2	6	4		3	2	8	8	42
France												105	95	200
Germany										50		50		100
Greece					3	15	12							30
Hungary							34	5		7	15			61
Ireland	2	2	3	2	5	1	1	1	3	4	4	2	1	31
Italy <sup>#</sup>					3	9	4	6	6	9	49	38	58	182
Malta								1	0		1		1	3
Netherlands						1		11	15	12	16	4	9	68
Norway	1	5	3	2	2	4	4	2	1	6	6	10	5	51
Portugal												20	18	38
Slovenia	1		1		2	1	1	0	0	0	0	0	0	6
Spain*				36	63	69	39	33	51	28	22	25	40	406
Sweden	6	5	4	2	15	13	13		1	13	8	15	22	117
United Kingdom										61	66	57	46	230
<b>Total</b>	<b>135</b>	<b>98</b>	<b>115</b>	<b>121</b>	<b>199</b>	<b>216</b>	<b>250</b>	<b>184</b>	<b>199</b>	<b>309</b>	<b>252</b>	<b>415*</b>	<b>376</b>	<b>2869</b>

\* Total includes 20 strains reported by Portugal and 35 strains reported by Bulgaria during the 2016 data collection. The number in the previous report was 360.

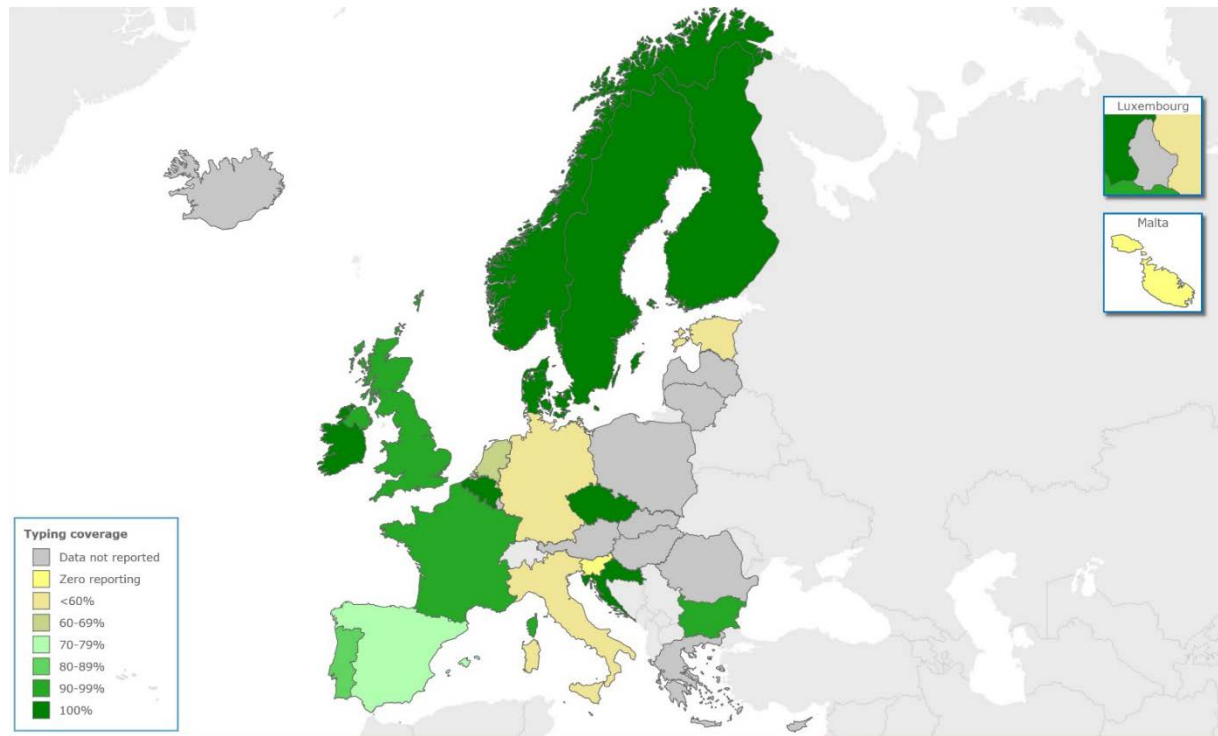
In 2015, the typing coverage was 90.4% (376/415 notified MDR TB cases) in the 17 Member States reporting molecular typing data for strains isolated. This ranged from 70.8% (Bulgaria) to 100% (Croatia, Denmark, Finland, Ireland, Malta, Norway, Portugal and Sweden) (Figure 1). In 2014, the typing coverage was 76.8% (415/540 notified MDR TB cases) in the 18 Member States reporting molecular typing data for strains isolated and this ranged from 48.7% (Italy) to 100% (Belgium, Croatia, Czech Republic, Denmark, Finland, Ireland, Norway and Sweden) (Figure 2).

**Figure 1. MDR TB typing coverage by country, EU/EEA, 2015**



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**Figure 2. MDR TB typing coverage by country, EU/EEA, 2014**



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## Clusters

Molecular typing data for MDR TB strains with full 24 loci MtbC15-9 code (<http://MIRUVNTRplus.org>) isolated in 2012–2015 were included in the cross-border molecular cluster analysis. A cross-border molecular cluster was defined as two or more *M. tuberculosis* isolates from at least two Member States (with at least one isolate from 2015) sharing identical complete MIRU-VNTR (24 loci) patterns, including any single missing locus shared by both, with no more than three years' difference between the years of isolation. Of 376 MDR TB strains isolated in 2015, 152 (40.4%) belonged to one of the 44 cross-border molecular clusters identified among strains isolated in 2012–2015. The size of those clusters varied between two and 170 strains, involving two to 16 Member States. The two most common MtbC15-9 types were 100-32 and 94-32, accounting for 30.7% (N=170) and 16.8% (N=93) of clustered strains isolated in the period 2012–2015 (Table 2).

Using the best match/similarity search algorithm on the MIRU-VNTRplus database ([www.miru-vntrplus.org](http://www.miru-vntrplus.org)), it was possible to assign a lineage (within 0.17 genetic distance) for 210 (55.8%) of the MDR TB strains isolated in 2015. More than half of the strains with assigned lineage belonged to the Beijing genotype (60.0%), followed by LAM, TUR, and Haarlem, with 17.6%, 9.0% and 5.2% respectively (Table 3).

**Table 2. Cross-border molecular MDR TB clusters involving strains isolated 2012–2015, by MtbC15-9 type**

MtbC15-9 type	Isolates 2012-2015 (n)	Countries involved (n)	Isolates by year (n)				Strain numbers by countries
			2012	2013	2014	2015	
100-32	170	11	45	39	50	36	Belgium (12), Estonia (84), Finland (6), France (21), Germany (13), Ireland (1), Italy (17), Norway (2), Spain (4), Sweden (4), United Kingdom (6)
94-32	93	16	19	13	38	23	Belgium (2), Bulgaria (2), Denmark (1), Estonia (12), Finland (3), France (19), Germany (22), Hungary (1), Ireland (1), Italy (12), Netherlands (3), Norway (2), Portugal (2), Spain (1), Sweden (5), United Kingdom (4)
11219-15	34	2	16	4	10	4	Bulgaria (33), Germany (1)
1065-32	29	7	9	7	5	8	Denmark (1), Estonia (9), France (7), Germany (1), Norway (1), Spain (3), United Kingdom (7)
843-52	20	9	4	3	8	5	Belgium (1), Estonia (4), France (5), Germany (3), Ireland (2), Netherlands (1), Norway (1), Spain (1), Sweden (2)
3554-15	18	7	4	1	6	7	Belgium (1), Denmark (1), Finland (1), France (1), Norway (2), Sweden (9), United Kingdom (3)
11219-82	14	2	2	4	7	1	Bulgaria (13), Denmark (1)
163-15	13	8	3	2	2	6	Bulgaria (1), France (1), Germany (1), Ireland (1), Italy (6), Norway (1), Portugal (1), Spain (1)
3182-15	13	5	1	4	1	7	France (2), Germany (2), Italy (5), Spain (3), United Kingdom (1)
1469-189	11	6	4	3	3	1	Belgium (2), Germany (1), Netherlands (2), Norway (2), Sweden (2), United Kingdom (2)
11327-26	9	3	2	3	2	2	Germany (1), Italy (6), Spain (2)
10571-67	8	2	1		2	5	Portugal (7), United Kingdom (1)
4737-32	8	4		1	6	1	Belgium (1), France (3), Italy (1), Portugal (3)
5298-54	8	2			4	4	France (1), Portugal (7)
1064-32	7	4	2	3	1	1	Netherlands (1), Norway (2), Sweden (3), United Kingdom (1)
1071-32	7	4	1		1	5	France (1), Germany (2), Italy (3), Spain (1)
342-32	6	3		4	1	1	France (1), Sweden (1), United Kingdom (4)
654-168	6	3	1		3	2	France (1), Italy (4), Spain (1)
11329-15	5	3	2	1		2	Finland (1), Norway (1), Sweden (3)
1773-32	5	4	2	1	1	1	Germany (1), Ireland (1), Netherlands (1), Sweden (2)
898-32	5	3	1	2	1	1	France (1), Germany (2), United Kingdom (2)
9183-463	5	2	2	1	1	1	Italy (4), Netherlands (1)
1066-32	4	2			1	3	Belgium (1), France (3)
1111-170	4	2	1	1	1	1	Finland (1), Netherlands (3)
3828-32	4	3		1	2	1	Belgium (1), Italy (1), Sweden (2)
94-15	4	2	2	1		1	Netherlands (3), Spain (1)
100-145	3	2	2			1	Belgium (1), Estonia (2)
1061-32	3	3			2	1	Germany (1), Norway (1), Sweden (1)
10975-903	3	3	1		1	1	France (1), Germany (1), Spain (3)
11062-15	3	2			2	1	France (2), Spain (1)
121-52	3	2		1	1	1	France (2), Spain (1)
2043-32	3	3	1			2	Germany (1), Portugal (1), Sweden (1)

MtbC15-9 type	Isolates 2012-2015 (n)	Countries involved (n)	Isolates by year (n)				Strain numbers by countries
			2012	2013	2014	2015	
67-25	3	2	1			2	Belgium (1), France (2)
1048-32	2	2		1		1	France (1), Netherlands (1)
10984-31	2	2			1	1	France (1), Italy (1)
11500-148	2	2		1		1	Sweden (1), United Kingdom (1)
13982-31	2	2			1	1	France (1), Spain (1)
15114-15	2	2		1		1	Italy (1), Spain (1)
1557-32	2	2		1		1	Denmark (1), Sweden (1)
17273-15	2	2			1	1	Germany (1), Spain (1)
357-15	2	2			1	1	Belgium (1), France (1)
698-15	2	2				2	Croatia (1), Spain (1)
7389-15	2	2				2	Norway (1), Sweden (1)
9354-32	2	2			1	1	France (1), Italy (1)

**Table 3. Number of genotypic lineages among MDR TB strains by country, EU/EEA, 2015**

Member State	Beijing	CAS	Ghana	Haarlem	LAM	S	TUR	URAL	X	Unknown	Total
Belgium	9			1	1		2				13
Bulgaria	1			1	9		2			4	17
Croatia										1	1
Czech Republic										8	8
Denmark	1						1			4	6
Estonia	19				1					8	28
Finland	4						1	1		2	8
France	30		3	2	15		1	1		43	95
Ireland										1	1
Italy	25				1			3		29	58
Malta										1	1
Netherlands	2				1					6	9
Norway							1			4	5
Portugal	1				4			1		12	18
Spain	9			5	2	4	7	1	1	11	40
Sweden	9	1		2			4			6	22
United Kingdom	16				3			1		26	46
<b>Total</b>	<b>126</b>	<b>1</b>	<b>3</b>	<b>11</b>	<b>37</b>	<b>4</b>	<b>19</b>	<b>8</b>	<b>1</b>	<b>166</b>	<b>376</b>

## Discussion

Based on the reported molecular typing data, no significant change in MDR TB epidemiology was observed compared to previous years. The MtbC15-9 types 100-32 and 94-32 remained the two most commonly identified in the EU/EEA and Beijing was the most common genotypic lineage (2-4).

However, the MIRU-VNTR 24 loci method does not have sufficient discriminatory powers to differentiate between strains within specific highly-conserved clusters including MtbC15-9 100-32 and 94-32. These data should therefore not be used to infer a large cross-border outbreak of MDR TB due to recent transmission. It would only be possible to identify actual recent cross-border transmission using typing methods with higher discriminatory powers (e.g. whole genome sequencing) complemented by epidemiological investigations (e.g. contact tracing).

Another important limitation of this report is the poor coverage of the EU/EEA. Only 18 of 31 Member States and two of the five high-priority MDR TB countries in the EU/EEA (Bulgaria, Estonia, Latvia, Lithuania, and Romania) reported data for 2015. The results may therefore not be representative for the whole EU/EEA.

Finally, epidemiological data to confirm chains of human transmission are largely missing. Epidemiological and molecular typing data can be linked by assigning an artificial unique strain code at national level that is reported together with the epidemiological data. However, this data linkage was only performed for 3% of MDR TB cases included in this report.

Due to the use of two different data sources (i.e. case-based and isolate-based) to compile the data presented this year, there may be some over- or under-reporting in the number and distribution of clustered cases compared to

the national data of individual countries. If you require up-to-date information about individual clusters, please contact the countries involved directly.

## Conclusions

The typing coverage of MDR TB strains at EU/EEA level remains suboptimal, and epidemiological and molecular typing data are only very rarely linked. Typing coverage at EU/EEA level could be improved by encouraging Member States to report the existing molecular typing data to TESSy and by promoting collaboration between the typing laboratories under the umbrella of the ECDC-funded European Laboratory Network for tuberculosis (ERLTB-Net).

Despite these limitations, EU/EEA level analysis of molecular typing data has identified strains possibly responsible for cross-border transmission of MDR TB that can be further investigated by the Member States involved to control the further spread of MDR TB.

## ECDC support

ECDC offers support to Member State experts in preparing their data, uploading them to TESSy and using the analysis tools for molecular typing data in TESSy. The supporting documents are available on the ECDC Tuberculosis Programme Extranet and on the TESSy web portal.

The ERLTB-Net offers training and capacity-building activities (e.g. exchange visits, twinning arrangements) as support to the Member States.

ECDC welcomes your feedback! Please send any comments or suggestions you may have to [csaba.kodmon@ecdc.europa.eu](mailto:csaba.kodmon@ecdc.europa.eu).

## Acknowledgements

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