SURVEILLANCE REPORT

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

January 2016

Summary

This report describes the geographical and temporal distribution of multidrug-resistant (MDR) tuberculosis (TB) based on molecular typing data as reported by European Union/European Economic Area (EU/EEA) Member States for 2014 and previous years.

Overall, 21 EU/EEA Member States reported 2 416 MDR TB isolates – all with a complete MIRU–VNTR profile – for the years 2003 to 2014 to The European Surveillance System (TESSy). In 2014, 17 Member States reported molecular typing data.

The number of isolates for which data were submitted increased from 238 in 2013 to 360 in 2014, and the typing coverage among all MDR TB strains increased from 16.0% in 2013 to 24.6% in 2014. In 2014, the typing coverage was below 50% in two Member States and at 100% in eight. Of 360 MDR TB strains isolated in 2014, 155 (43.1%) belonged to one of the 35 cross-border molecular clusters of MDR TB strains that were isolated in 2011–2014 and assigned an MtbC15-9 type. The cluster size varied between 2 and 182 strains, involving between 2 and 14 Member States. The two most common MtbC15-9 types were 100-32 and 94-32, which accounted for 33.6% and 23.7% of clustered isolates in 2014, respectively. A lineage was assigned for 70.8% of MDR TB strains isolated in 2014; more than half of them belonged to the Beijing genotype (62.6%), followed by LAM, Haarlem, URAL and TUR genotypes.

Typing coverage at the EU/EEA level remains suboptimal. Also, the representativeness of typing data is not optimal, especially because out of the five EU/EEA high-priority MDR TB countries (Bulgaria, Estonia, Latvia, Lithuania, and Romania), only Estonia reported data for 2014.

Despite its current limitations, the analysis of molecular typing data at the EU/EEA level is useful to identify strains that may be responsible for the cross-border transmission of MDR TB. These strains can then be further investigated by the Member States in order to control the further spread of MDR TB.

Background

24-locus MIRU–VNTR genotyping (mycobacterial interspersed repetitive units (MIRU)–variable number of tandem repeats (VNTR)) has become the standard tool for molecular typing of *Mycobacterium tuberculosis* strains in

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tuberculosis (TB) control programmes in many European Union/European Economic Area (EU/EEA) Member States. From 2009 till 2012, ECDC funded a project on the molecular typing for surveillance of the international transmission of MDR TB and XDR TB in the EU/EEA, which was outsourced to the Tuberculosis Reference Laboratory at the Dutch National Institute for Public Health and the Environment, Bilthoven, the Netherlands. In January 2013, ECDC initiated a pilot study to gain first-hand experiences with the collection and analysis of molecular typing data at the EU/EEA level and to assess the benefits and challenges of this method and its resource needs. The study was limited in scope (*Salmonella*, *Listeria*, Shiga toxin-/verocytotoxin-producing *E. coli* and MDR TB) and in time (January 2013 to May 2014). It enabled ECDC and the Member States to test the technical platform on a manageable scale. The pilot evaluation revealed a need to improve the functioning, flexibility and user-friendliness of the tools for data submission, cluster visualisation and analysis at the EU/EEA level. The assessment of the public health usefulness of EU-level molecular typing for surveillance was limited by the timeframe of the pilot, but participants recognised the relevance of molecular typing for surveillance of MDR TB at the EU level.

The main recommendation of the ECDC evaluation of multidrug-resistant (MDR) TB molecular typing for surveillance was to continue collecting MDR TB molecular typing data through TESSy, but not on a real-time basis because the long reporting delay and the small proportion of MDR TB isolates genotyped in some Member States did not justify a monthly cluster analyses. Instead, it was recommended that the submission of MDR TB isolate data should be integrated into the annual TB data reporting to TESSy, so that both isolate- and case-based data are submitted at the same time – or at least within the same timeframe. A network expert group was established to support ECDC in analysing the data annually and produce a status report on the dynamics, geographic distribution and trends of MDR TB strains and clusters in Europe.

This is the first annual report which focuses on strains isolated in 2014.

Overview

Data

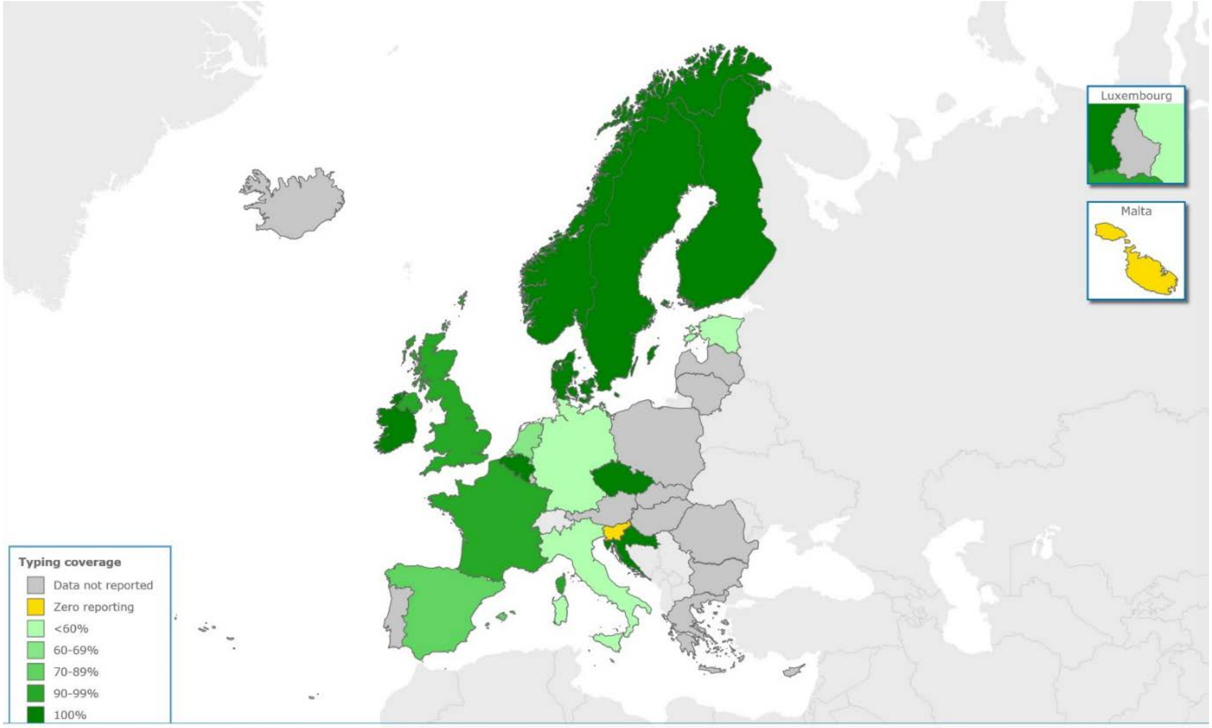
Data pertaining to MDR TB strains isolated from 2003 to 2014 were extracted from TESSy on 7 December 2015. During this period, 2 416 MDR TB isolates with complete MIRU-VNTR 24 loci data were reported by 21 countries (Table 1). In 2014, data were reported for 360 MDR TB strains by 17 Member States, including two countries with zero reporting (Malta and Slovenia). In 2014, France started reporting molecular typing data to TESSy, accounting for 29.2% of all submitted isolates in 2014. Altogether, typing data were reported for 24.6% of 1 463 MDR TB cases notified in the EU/EEA in 2014. The typing coverage increased from 16.0% in 2013 (Table 1) [1]. Typing coverage was 78.4% (360/481 notified MDR TB cases) for the 17 Member States reporting molecular typing data and ranged from 48.7% (Italy) to 100% (Belgium, Croatia, the Czech Republic, Denmark, Finland, Ireland, Norway and Sweden) (Figure 1). However, Italian data are provisional as molecular typing data for 29 strains isolated in 2014 were not available at the time of data extraction for this report.

Table 1. **Number of MDR TB strains typed by MIRU-VNTR (24-loci) and reported to TESSy, EU/EEA, 2003–2014**

Country	Year												Total
	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	
Belgium							10	20	14	22	12	13	91
Bulgaria					20	20	38	42	45	34	18		217
Croatia	15	5	7	11	3	3	6	3	2	2	0	2	59
Cyprus						1							1
Czech Republic	6	2	6	5	1	9	6			4	0	5	44
Denmark	0	0	5	3	2	2	2	2	3	1	2	2	24
Estonia	101	79	84	58	78	66	74	54	58	53	31	24	760
Finland	3		2	2	2	2	6	4		3	2	8	34
France												105	105
Germany										50		50	100
Greece					3	15	12						30
Hungary							34	5					39
Ireland	2	2	3	2	5	1	1	1	3	4	4	2	30
Italy#					3	9	4	6	6	9	49	38	124
Malta								1	0	0	1	0	2
Netherlands						1		11	15	11	17	4	59
Norway	1	5	3	2	2	4	4	2	1	6	6	10	46
Slovenia	1		1		2	1	1			0	0	0	6
Spain*				36	63	69	39	33	51	28	22	25	366
Sweden	6	5	4	2	15	13	13		1	13	8	15	95
United Kingdom										61	66	57	184
Total	**135**	**98**	**115**	**121**	**199**	**216**	**250**	**184**	**199**	**301**	**238**	**360**	**2416**

 # Data for 29 strains isolated in 2014 not included.
** Typing data for two strains isolated in 2014 not included.*

Figure 1. **MDR TB typing coverage by country, 2014**



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Clusters

Molecular typing data for MDR TB strains with completely assigned MtbC15-9 code[1] (http://MIRUVNTRplus.org) isolated in 2011–2014 were included into 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 (and including at least one isolate from 2014) that share 100% matching MIRU-VNTR (24 loci) patterns including any single missing locus shared by both and no more than three years difference in 'year of isolation' between two isolates. Of 360 MDR TB strains isolated in 2014, 155 (43.1%) belonged to one of the 35 cross-border molecular clusters that were identified between 2011 and 2014 and assigned an MtbC15-9 type. The size of those clusters varied between 2 and 182 strains, involving between 2 and 14 Member States (Table 2).

Table 2. **Cross-border molecular MDR TB clusters involving strains isolated in 2014, by MtbC15-9 type**

MtbC15-9 type	Strains	Countries involved	Strain numbers by countries
100-32	182	11	Belgium (9), Estonia (114), Finland (3), France (15), Germany (13), Ireland (2), Italy (12), Norway (2), Spain (4), Sweden (2), United Kingdom (6)
94-32	73	14	Belgium (3), Bulgaria (1), Denmark (1), Estonia (10), Finland (2), France (17), Germany (22), Ireland (1), Italy (4), Netherlands (2), Norway (2), Spain (1), Sweden (3), United Kingdom (4)
1065-32	21	5	Estonia (9), France (3), Germany (1), Norway (1), United Kingdom (7)
843-52	20	8	Belgium (2), Estonia (4), France (3), Germany (3), Ireland (2), Norway (1), Spain (3), Sweden (2)
3554-15	11	4	Belgium (1), Norway (1), Sweden (6), United Kingdom (3)
1469-189	10	6	Belgium (2), Germany (1), Netherlands (2), Norway (2), Sweden (1), United Kingdom (2)
163-15	8	5	Bulgaria (1), Germany (1), Ireland (2), Italy (3), Norway (1)
1064-32	8	5	Belgium (1), Netherlands (2), Norway (1), Sweden (3), United Kingdom (1)
11327-26	8	3	Germany (1), Italy (6), Spain (1)
342-32	6	3	France (1), Spain (1), United Kingdom (4)
3182-15	6	3	Germany (2), Italy (3), United Kingdom (1)
4737-32	5	3	Belgium (1), France (3), Italy (1)
407-32	5	4	Estonia (1), France (1), Italy (1), Netherlands (2)
1773-32	4	4	Germany (1), Ireland (1), Netherlands (1), Sweden (1)
3828-32	4	4	Estonia (1), Italy (1), Netherlands (1), Sweden (1)
14747-31	4	4	Germany (1), Italy (1), Spain (1), United Kingdom (1)
9183-463	4	2	Italy (3), Netherlands (1)
10975-903	4	2	Germany (1), Spain (3)

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[1] 'The MtbC15-9 type is defined by combination of a type based on the 15 loci (MtbC15) of the discriminatory subset and a type based on the 9 auxiliary loci (MtbC9). The MtbC15-9 type is displayed as a combination, e.g. 11-22, where "11" is the MtbC15 type and "22" is the MtbC9 type.' [4]

MtbC15-9 type	Strains	Countries involved	Strain numbers by countries
898-32	4	2	Germany (2), United Kingdom (2)
11427-32	4	2	France (3), Italy (1)
654-168	4	2	Italy (3), Spain (1)
11419-85	3	2	France (2), Spain (1)
166-26	3	2	France (1), Spain (2)
1111-170	3	2	Finland (1), Netherlands (2)
677-66	2	2	France (1), Netherlands (1)
14951-31	2	2	France (1), Italy (1)
1076-32	2	2	Belgium (1), Germany (1)
1061-32	2	2	Germany (1), Norway (1)
9115-384	2	2	Denmark (1), Norway (1)
95-32	2	2	Bulgaria (1), Estonia (1)
10984-31	2	2	France (1), Spain (1)
121-52	2	2	France (1), Spain (1)
11417-31	2	2	France (1), Italy (1)
357-15	2	2	Belgium (1), France (1)
95-69	2	2	Germany (1), Norway (1)

The two most common MtbC15-9 types were 100-32 and 94-32, which accounted for 33.6% (N=51) and 23.7% (N=36) of clustered strains, respectively. Cluster 94-32 showed an increasing trend in number of isolates during the period under surveillance. This increase may be due to a higher typing coverage in 2014 compared to previous years and/or to recent transmission (Table 3). Discriminatory power of the MIRU-VNTR 24 loci method is not high enough to differentiate strains within some specific clusters; in particular, MtbC15-9 100-32 and 94-32. For this reason it should not be concluded that a large cross-border outbreak of MDR TB has occurred in the EU/EEA in 2014. Actual cross-border transmission could only be identified by using typing methods with higher discriminatory power (e.g. whole genome sequencing) and by performing epidemiological investigations (e.g. contact tracing).

Table 3. Cross-border molecular MDR TB clusters involving strains isolated in 2014, by MtbC15-9 type and year, 2011-2014

MtbC15-9 type	Year				Total
	2011	2012	2013	2014	
100-32	47	45	39	51	182
94-32	6	19	12	36	73
1065-32		9	7	5	21
843-52	5	4	3	8	20
3554-15		4	1	6	11
1469-189		4	3	3	10
163-15	1	3	2	2	8
1064-32	2	2	3	1	8
11327-26	1	2	3	2	8
342-32	1		4	1	6
3182-15		1	4	1	6
4737-32	1		1	3	5
407-32	1			4	5
1773-32		2	1	1	4
9183-463		2	1	1	4
3828-32	1		1	2	4
898-32		1	2	1	4
11427-32			1	3	4
654-168		1		3	4
14747-31		1	1	2	4
10975-903	2	1		1	4
11419-85		1		2	3
166-26	2			1	3
1111-170		1	1	1	3
677-66			1	1	2
14951-31	1			1	2
1076-32		1		1	2
1061-32				2	2
9115-384	1			1	2
95-32	1			1	2
10984-31	1			1	2
121-52			1	1	2

MtbC15-9 type	Year				Total
	2011	2012	2013	2014	
11417-31			1	1	2
357-15	1			1	2
95-69		1		1	2

Using the best match/similarity search algorithm on the MIRU-VNTRplus database (www.miru-vntrplus.org), it was possible to assign a lineage (within 0.17 genetic distance) for 255 (70.8%) of the MDR TB strains isolated in 2014; of these 255 isolates, 92 (36.1%) were found to be a 100% match. More than half of the strains with assigned lineage belonged to the Beijing genotype (62.6%), followed by LAM (10.6%), Haarlem (9.4%), URAL (3.9%) and TUR (3.9%) genotypes (Table 4). Two strains were defined as *M. africanum* (West African I sublineage).

Table 4. Number of genotypic lineages among MDR TB strains by country, 2014

Member State	Beijing	Cameroon	Haarlem	LAM	TUR	Delhi CAS	Ghana	URAL	Uganda II	X	EAI	Africanum	Unknown	Total
Belgium	4		1		1								7	3
Croatia			1								1			2
Czech Republic	2			2									1	5
Denmark													2	2
Estonia	21			3										24
Finland	4										1		3	8
France	51		7	12			3	3	2	1	3		23	105
Germany	34	1	1	4				1					9	50
Ireland				2										2
Italy	7	1	8	1				3		1			17	38
Netherlands	1												3	4
Norway	5				2						1		2	10
Spain	2		5	1				1	1			1	14	25
Sweden	6				5						1	1	2	15
United Kingdom	22	3	1	2	2	1		2			2		22	57
Total	**159**	**5**	**24**	**27**	**10**	**1**	**3**	**10**	**3**	**2**	**9**	**2**	**105**	**360**

Of 360 MDR TB strains, second-line drug susceptibility data were reported for 307 (85.1%), of which 46 (15.0%) were extensively drug-resistant (XDR). For 41 XDR TB strains, the complete MtbC15-9 code was assigned and 32 (78.0%) belonged to five clusters. The 100-32 and 94-32 clusters consisted of 13 and 12 strains, respectively, followed by 11427-32, 1065-32 and 407-32, with 3, 2 and 2 strains, respectively.

In the period 2011–2014, complete MIRU-VNTR (24 loci) typing data of 382 (34.9%) strains isolated from patients of foreign origin were reported. A total of 177 (16.2%) strains were from native patients. For the remaining strains (N=536; 48.9%), the origin was not reported. Among strains isolated from patients of foreign origin, 172 (45.0%) belonged to one of 24 cross-border clusters. Among strains isolated from patients of native origin, 10 (5.6%) belonged to one of four cross-border clusters. In the same period, Member States included in this analysis reported 964 (73.4%) MDR TB cases in individuals of foreign origin to the surveillance database. They also reported 329 (25.1%) MDR TB cases among individuals of native origin and an additional 20 (1.5%) MDR TB cases without information on origin [1].

Discussion

Compared to 2013, the number of Member States providing molecular typing data increased by one. Data reported for previous years were updated by Estonia, Sweden and the United Kingdom. The number of isolates for which data were submitted increased from 238 in 2013 to 360 in 2014.

Typing coverage among all isolated MDR TB strains increased from 16.0% in 2013 to 24.6% in 2014. In two Member States, the typing coverage was below 50%, and in eight the typing coverage was 100%. Almost half (42.2%) of the MDR TB strains isolated in 2014 and included in this analysis belonged to one of 35 cross-border clusters identified. The largest clusters were MtbC15-9: 100-32 and 94-32, where 94-32 showed an increase in number of isolates over the years. These results are consistent with observations described elsewhere [2]. In 2014, the most frequently identified lineage was Beijing, which is very common among MDR TB isolates in Estonia [2] and foreign-born MDR TB cases from areas that already show a high prevalence of Beijing lineage [1,3].

Conclusions

Despite an observed increase, typing coverage at the EU/EEA level remains suboptimal. Out of the five high-priority MDR TB countries (Bulgaria, Estonia, Latvia, Lithuania, and Romania), only Estonia reported data for 2014. As a result, molecular typing data presented in this document cannot be considered representative for the whole EU/EEA.

The low completeness of data for some variables (e.g. country of birth/nationality, resistance results for second-line anti-TB drugs) does not allow in-depth analysis of cluster dynamics, possible transmission routes and the proportion of XDR TB within clusters.

Despite its current limitations, the analysis of molecular typing data at the EU/EEA level is useful to identify strains that may be responsible for the cross-border transmission of MDR TB. These strains can then be further investigated by the Member States in order 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.

ECDC welcomes your feedback. Please send any comments or suggestions you may have to csaba.kodmon@ecdc.europa.eu.

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