Tuberculosis molecular surveillance status report, focussing on rifampicin and multi-drug resistance in the EU/EEA

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Summary

Between 2020 and 2023, the European Centre for Disease Prevention and Control (ECDC) analysed whole genome sequencing (WGS) profiles of rifampicin resistant (RR)- and multi-drug resistant (MDR) *Mycobacterium tuberculosis* strains isolated in the European Union (EU) and European Economic Area (EEA). The results obtained were added to and compared with tuberculosis (TB) WGS data collected during the period 2017−2019 as a part of an EU/EEA pilot project on TB molecular surveillance.

Analysis of 2 490 RR/MDR TB isolates with complete WGS data showed:

- a sub-optimal typing coverage at EU/EEA level. Only nine countries contributed, with 514 strain sequences during the period 2020−2022;
- wide variation in the sequencing coverage. The highest sequencing coverage was achieved in 2018 (71.0%), while the lowest coverage was observed in 2020 (18.8%);
- a relatively stable clustering proportion. The lowest clustering percentage was observed in 2018 (53.9%) and the highest in 2020 and 2021 (64.1%);
- the identification of 67 cross-border molecular clusters, comprising 632 RR/MDR TB strains in total. Only 18 clusters included 10 or more strains, involving two to nine countries in each cluster;
- limitations due to the incompleteness of data for some non-mandatory variables (e.g. country of birth/nationality). As a consequence, in-depth analyses of cluster dynamics and possible transmission routes within the clusters was not possible;
- successful lineage assignment in 87.5% of the strains. The most prevalent lineages were Beijing (N=749, 30.1%), Mainly T (N=534, 21.4%) and Haarlem (N=303, 12.2%). Due to the low number of countries submitting WGS data during the period 2020−2022, Beijing lineage became a dominant strain, however this should be interpreted with caution.

The COVID-19 pandemic had a significant impact on TB laboratory services in the EU/EEA [1]. In acknowledgement of this fact, and so as to continue supporting EU/EEA countries in consolidating TB WGS-based surveillance activities, ECDC offered sequencing support to countries wishing to have their RR/MDR TB strains sequenced. Six countries showed interest and benefited from this support. A further three countries with in-country WGS capacity also submitted WGS data during the period 2020−2022.

In conclusion, despite the support and IT infrastructure available at ECDC, efforts are still needed to consolidate the WGS-based surveillance of TB in the EU/EEA in order to achieve the 2017−2019 level as a minimum. A higher coverage of WGS will contribute to a better understanding of rifampicin resistant (RR)- and multi-drug resistant (MDR) TB strain diversity, early detection and tracing of trans-national outbreaks and the mapping of transmission routes across Europe, as well as changes in the resistance pattern and mechanism.

Background

Genotyping has become a standard tool in tuberculosis (TB) control programmes for many countries within the European Union (EU) and European Economic Area (EEA). Thanks to its discriminatory power, offering the ultimate resolution currently achievable, WGS is gaining recognition as the gold standard for TB transmission studies and outbreak investigations. Approximately half of the TB reference laboratories in the EU/EEA are using WGS to characterise drug resistance profiles and to assess the genetic relatedness of the *Mycobacterium tuberculosis* (MTB) strains isolated at country level [2].

In September 2017, the European Centre for Disease Prevention and Control (ECDC) initiated a 30-month (2017−2020) pilot project on the use of WGS for molecular typing and characterisation of *Mycobacterium tuberculosis* complex (MTBC) isolates in the EU/EEA. This pilot project generated evidence on the use of WGS for a better understanding of RR/MDR TB strain diversity, early detection and tracing of trans-national outbreaks and the mapping of transmission routes across Europe. In addition, analysis and reporting standards were established, along with WGS methodology standards to ensure data comparability within EU/EEA [3].

The pilot project showed that a WGS-based surveillance system is not only feasible but can efficiently elucidate the dynamics of in-country and cross-border RR/MDR-TB transmission across EU/EEA countries. Lessons learned from this study highlight that the establishment of an EU/EEA centralised WGS-based surveillance system for TB will require the strengthening of national integrated systems (including WGS, microbiology and epidemiological data) performing prospective WGS surveillance. In addition, the development of clear procedures to facilitate international collaboration for the investigation of cross-border clusters will also be necessary. Therefore, ECDC decided to introduce WGS-based surveillance into routine TB surveillance activities and developed the TB module on the EpiPulse[1] platform for analysis and visualisation of TB WGS data.

Building upon the lessons learned from the pilot WGS project, ECDC has provided additional technical support to EU/EEA countries interested in using WGS for molecular characterisation of RR/MDR TB strains isolated between 2020 and 2022. This support included the provision of sequencing services for the EU/EEA countries without available capacity for WGS, as well as online support with data uploads to The European Surveillance System (TESSy) and data analysis in EpiPulse. All WGS results obtained as a result of this support were included in TESSy.

In addition, ECDC requested EU/EEA countries performing routine WGS for RR/MDR TB strains to submit the WGS data of RR/MDR TB strains isolated between 2020 and 2022 to TESSy, as first step towards a systematic TB molecular surveillance.

Aim

The aim of this report is to provide an overview of the dynamics and geographical distribution of RR/MDR TB strains in the EU/EEA, focussing on the strains isolated in 2020−2022, and to compare this with WGS data collected in 2017−2019 as a part of an EU/EEA pilot project on TB molecular surveillance.

Overview

Data

The isolate data were extracted from TESSy on 26 May 2023. The database contains 2 490 RR/MDR TB isolates with complete WGS data reported during the period 2017−2022. During the period covered by the EUMySeqTB project (2017−2019), 23 countries submitted 1 976 sequences to TESSy, while during the period 2020−2022 only 514 sequences were submitted by nine countries (Table 1). The data suggest that the COVID-19 pandemic had a significant impact on TB laboratory services and WGS data reporting.

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[1] ECDC's EpiPulse platform is available at: https://epipulse.ecdc.europa.eu

Table 1. **Number of RR/MDR TB strains with WGS data reported to TESSy, 2017−2022**

Country	Year						Total
	2017	2018	2019	2020	2021	2022	
Austria		17	6				23
Belgium	5	8	6				19
Bulgaria	24	24	16				64
Croatia		2	1				3
Czechia	5	8	11	3	11		38
Denmark		4	3				7
Estonia	29	28	24	21	21	7	130
Finland	4	4	2				10
France	8	61	25				94
Germany	70	93	15				178
Hungary	3	12	4	13	16	13	61
Ireland	6	6	8				20
Italy	63	40	20	21	26	5	175
Latvia	18	33	33				84
Lithuania	80	63	52	39	48	66	348
Netherlands	10	6	6			8	30
Norway	3	6	2				11
Poland	27	54	10	41	51	68	251
Portugal	12	19	4				35
Romania	306	300	174				780
Slovakia	1	3	2				6
Slovenia	1						1
Spain	15	35	12	2	3	3	70
Sweden	9	10	5	7	7	14	52
Total	**699**	**836**	**441**	**147**	**183**	**184**	**2 490**

The TB notification data was extracted from TESSy on 26 May 2023. The data for 2022 (from 14 countries) were incomplete. During the period 2017−2021, the number of notified RR/MDR TB cases showed a decreasing trend from 1 332 to 770 in 2021, following the trend in TB notifications [4]. The highest sequencing coverage was achieved in 2018 (71.0%), while the lowest coverage was observed in 2020 (18.8%). The lowest clustering percentage was observed in 2018 (53.9%) and the highest in 2020 (64.1%).

Figure 1. **Sequencing coverage and clustering percentage of RR/MDR tuberculosis, EU/EEA, 2017−2022***
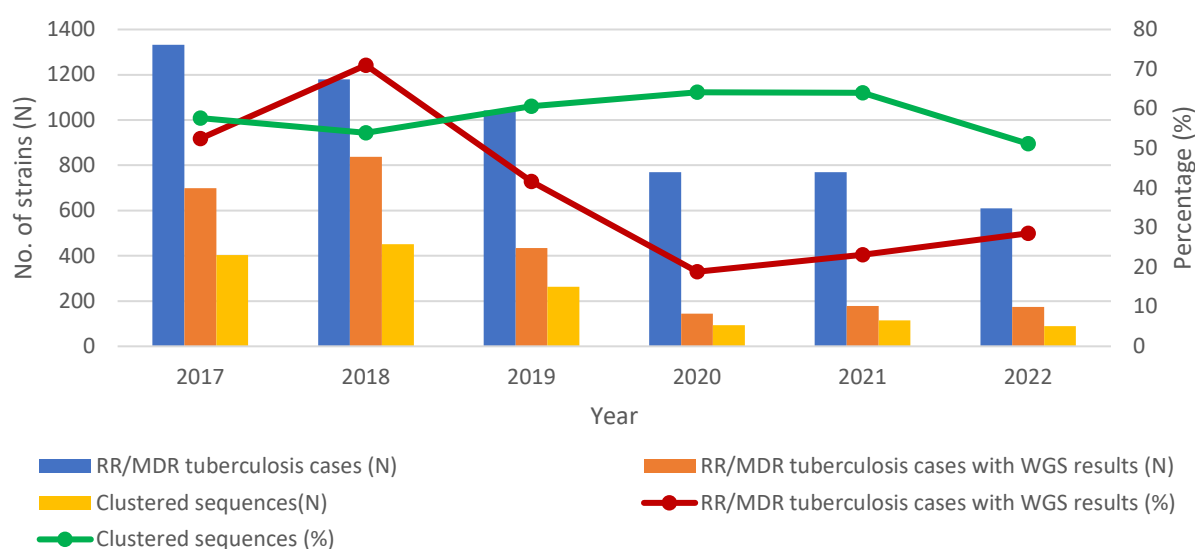
The 2022 RR/MDR TB notification data are incomplete.

Clusters

The cluster identification was performed using the ECDC pipeline by comparing the 2 891 Core Genome Multi-locus Sequence Typing (cgMLST) loci. A cross-border molecular cluster was defined as two or more *M. tuberculosis* isolates from at least two EU/EEA Member States with less than six allelic differences (AD). In this analysis the emphasis was on cross-border molecular clusters which included at least one isolate from 2020 or later.

Among the RR/MDR TB strains covering the period from 2017 till 2022, 67 cross-border molecular clusters were detected and a total of 632 RR/MDR TB strains were clustered. The cluster size varied between two and 71 strains involving two to nine countries. Only 18 clusters included 10 or more strains in total (Table 2).

Table 2. **Cross-border molecular clusters with strains isolated during the period 2020−2022**

Cluster ID	Number of isolates	Countries
2021-09.TB.17.Beijing	71	EE(22), LT(44), DE(4), HU(1)
2021-09.TB.41.Beijing	63	PL(48), FR(2), BE(1), CZ(3), IT(5), SK(1), NL(1), DE(1), EE(1)
2021-09.TB.11.mainlyT	35	RO(17), IT(18)
2021-09.TB.37.mainlyT	35	RO(31), AT(1), IT(3)
2021-09.TB.31.Beijing	30	LT(26), IE(2), PL(2)
2021-09.TB.214.Beijing	25	PL(19), DE(2), IT(2), LT(1), SE(1)
2021-09.TB.46.LAM	25	LT(26), NO(1)
2021-09.TB.10.Beijing	19	PL(1), LT(15), DE(1), CZ(1), SE(1)
2021-09.TB.50.Ural	19	RO(18), DE(1)
2021-09.TB.49.mainlyT	18	RO(16), DE(1), BE(1)
2021-09.TB.120.Euro-American	16	DE(7), FR(1), IT(4), AT(4)
2021-09.TB.57.Beijing	16	LV(1), LT(4), DE(5), PL(4), IT(1), CZ(1)
2021-09.TB.24.mainlyT	14	RO(13), DE(1)
2021-09.TB.55.Beijing	14	CZ(3), PL(5), EE(1), SE(3), ES(1), DE(1)
2021-09.TB.66.Haarlem	13	RO(11), IT(2)
2021-09.TB.09.Euro-American	12	DE(5), IT(5), SE(1), NL(1)
2021-09.TB.153.X-type	10	ES(9), SE(1)
2021-09.TB.19.mainlyT	10	RO(9), AT(1)
2021-09.TB.23.Euro-American	9	IT(3), DE(1), FR(2), SE(3)
2021-09.TB.234.H37Rv-like	9	HU(8), IT(1)
2023-04.TB.29.Beijing (2.2.1)	9	DK(1), AT(1), PL(6), CZ(1)
2021-09.TB.03.LAM	8	IT(5), ES(3)
2021-09.TB.26.Ural	8	PT(1), DE(2), IT(4), RO(1)
2021-09.TB.43.LAM	8	LV(4), FR(1), NL(1), EE(2)
2021-09.TB.169.LAM	7	LV(5), IE(1), LT(1)
2021-09.TB.38.Beijing	7	DE(3), IT(1), FR(1), AT(1), SE(1)
2021-09.TB.206.Ural	6	ES(3), LT(3)
2021-09.TB.45.Beijing	6	AT(3), DE(2), SE(1)
2021-09.TB.109.Haarlem	5	IT(1), RO(4)
2021-09.TB.280.Beijing	5	BE(2), PL(3)
2021-09.TB.33.Ural	5	IE(1), LT(4)
2021-09.TB.29.LAM	5	ES(1), NL(1), IT(3)
2021-09.TB.79.Haarlem	5	FR(1), RO(4)
2021-09.TB.89.TUR	5	BG(4), SI(1)
2021-09.TB.207.Ural	4	PL(3), DE(1)
2021-09.TB.266.TUR	4	DE(2), BG(2)
2021-09.TB.47.mainlyT	4	RO(3), DE(1)
2021-09.TB.116.Beijing	3	SE(1), IT(1), PL(1)
2021-09.TB.15.mainlyT	3	IT(1), RO(2)
2021-09.TB.168.mainlyT	3	RO(2), DE(1)
2023-04.TB.32.Mainly T (4.7)	3	HR(1), HU(2)
2021-09.TB.245.Haarlem	3	NO(1), RO(2)
2021-09.TB.277.Beijing	3	IT(2), SE(1)
2021-09.TB.30.Euro-American	3	IT(1), DE(1), FR(1)
2021-09.TB.90.mainlyT	3	RO(2), IT(1)
2021-09.TB.100.Euro-American	2	NL(1), PL(1)
2021-09.TB.111.Delhi-CAS	2	FI(1), SE(1)
2021-09.TB.122.mainlyT	2	RO(1), IT(1)
2021-09.TB.140.LAM	2	FR(1), ES(1)
2021-09.TB.159.LAM	2	BE(1), ES(1)

Cluster ID	Number of isolates	Countries
2021-09.TB.161.Haarlem	2	IT(1), RO(1)
2021-09.TB.251.Beijing	2	FR(1), IT(1)
2021-09.TB.264.Euro-American	2	IT(1), DE(1)
2021-09.TB.290.Beijing	2	NL(1), LT(1)
2021-09.TB.293.LAM	2	ES(1), PT(1)
2021-09.TB.296.Beijing	2	FR(1), NL(1)
2021-09.TB.61.mainlyT	2	BG(1), DE(1)
2021-09.TB.75.Beijing	2	DE(1), LT(1)
2021-09.TB.82.mainlyT	2	FR(1), BG(1)
2021-09.TB.97.Ural	2	SK(1), PL(1)
2023-04.TB.16.Beijing (2.2.1)	2	DE(1), PL(1)
2023-04.TB.12.Beijing (2.2.1)	2	CZ(1), PL(1)
2023-04.TB.21.Beijing (2.2.1)	2	LT(1), NL(1)
2023-04.TB.26.Haarlem (4.1.2.1)	2	DE(1), FR(1)
2023-04.TB.41.Beijing (2.2.1)	2	DE(1), IT(1)
2023-04.TB.42.Mainly T (4.8)	2	RO(1), PL(1)
2023-04.TB.44.Beijing (2.2.1)	2	AT(1), HU(1)

Of 2 490 RR/MDR TB strains, 2 179 (87.5%) had a successful lineage assignment. The most prevalent lineages were Beijing (N=749, 30.1%), Mainly T (N=534, 21.4%) and Haarlem (N=303, 12.2%) (Table 3).

Table 3. **Genotypic lineages of RR/MDR TB strains by year**

Lineage	Year						Total
	2017	2018	2019	2020	2021	2022	
Beijing	209	255	127	51	53	54	749
MainlyT	176	225	119	6	7	1	534
Haarlem	125	106	68	1	3		303
LAM	53	88	33	8	9	4	195
Ural	36	34	33	4	9	8	124
Euro-American	36	46	20	1	3	1	107
S-type	20	13	4				37
TUR	15	16	6				37
Delhi-CAS	11	18	7				36
X-type	1	9	2	2	2	3	19
EAI	5	9	4				18
H37Rv-like	3	2	1	3	2	1	12
Cameroon	5						5
East-Asian			1				1
Uganda			1				1
West-Africa		1					1
Not assigned	4	14	15	71	95	112	311
Total	**699**	**836**	**441**	**147**	**183**	**184**	**2 490**

The three most prevalent lineages are not equally distributed across the EU/EEA. During the period 2017−2019, Haarlem and Mainly T were most prevalent in Romania which reported 245 (81.9%) of the 299 Haarlem strains reported in the EU/EEA, and 418 (80.4%) of the Mainly T strains. During the same period strains belonging to Beijing lineage were more equally distributed across the EU/EEA (Figure 2). In the period 2020−2022, Beijing lineage became a dominant strain, but this should be interpreted with caution due to the low number of countries submitting WGS data during that period (Figure 3).

Figure 2. Beijing, Haarlem and Mainly T lineages isolated in 2017−2019

Beijing ■ Haarlem ■ Mainly T
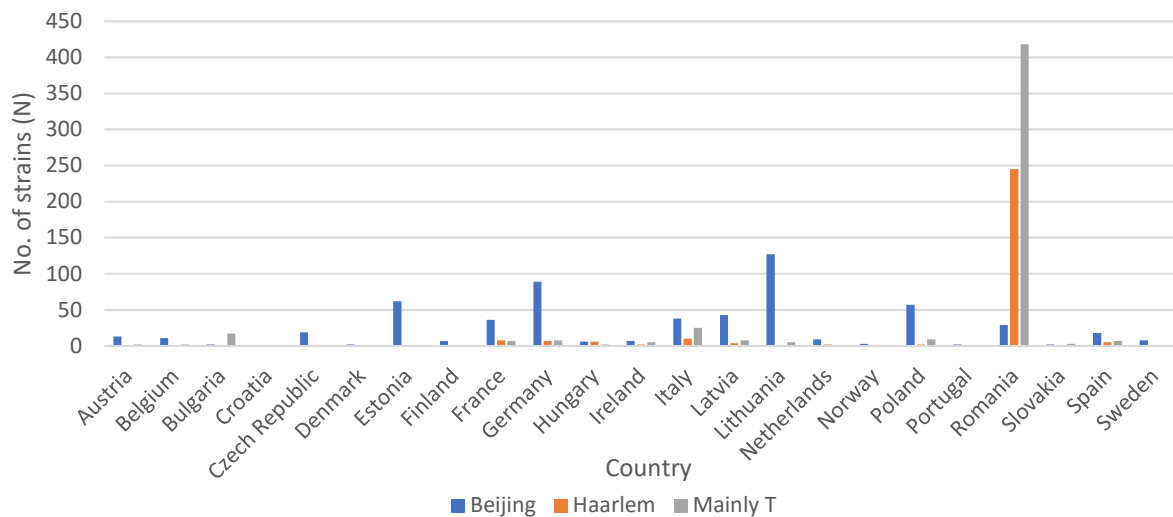
Figure 3. Beijing, Haarlem and Mainly T lineages isolated in 2020−2022

Beijing ■ Haarlem ■ Mainly T
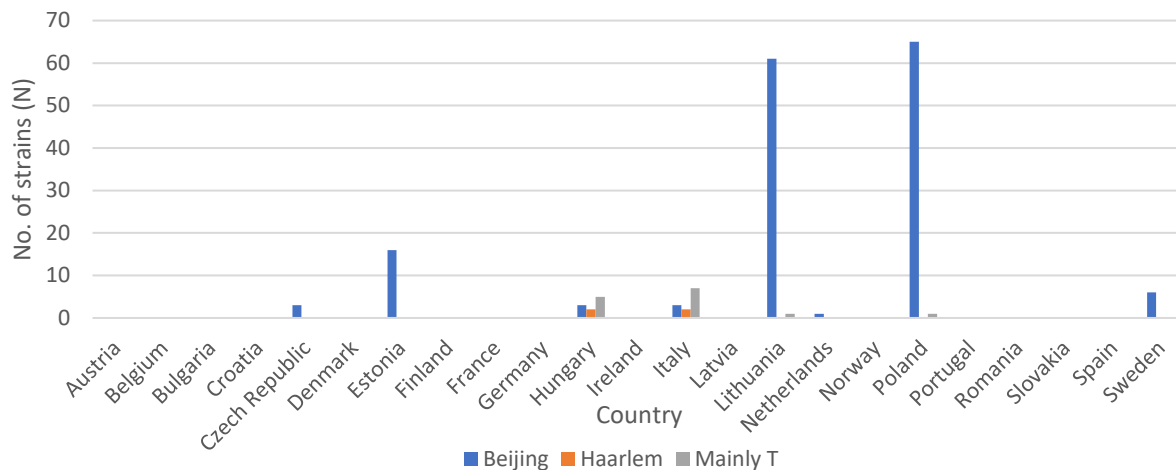
Conclusions

The COVID-19 pandemic had a significant impact on TB laboratory services in the EU/EEA [1]. The sequencing coverage at EU/EEA level was sub-optimal during the period 2020−2022, and the data completeness for some variables (e.g. country of birth/nationality) needs to be improved. Sequencing coverage should at least reach the level of the 2017−2019 period in order to allow for in-depth analysis of cluster dynamics and possible transmission routes within the EU/EEA. Therefore, further efforts are needed in the countries, along with support from ECDC and other EU initiatives in order to consolidate TB WGS-based surveillance activities in the EU/EEA.

Next steps

ECDC will continue to provide support to the EU/EEA Member States for the sequencing of RR/MDR TB strains, data reporting to the molecular surveillance module of TESSy and the use of EpiPulse for analysis and visualisation of data.

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

Acknowledgments

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