Molecular and genomic typing for tuberculosis surveillance: A survey study in 26 European countries

Marta Andrés¹, Marieke J. van der Werf², Csaba Ködmön³, Stefan Albrecht¹, Walter Haas¹, Lena Fiebig¹*, Survey study group

¹ Department of Infectious Disease Epidemiology, Robert Koch Institute, Berlin, Germany, ² European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden

Membership list can be found in the Acknowledgments section

* lena.fiebig@apo.org, tuberkulose@rki.de

Abstract

Background

Molecular typing and whole genome sequencing (WGS) information is used for (inter-)national outbreak investigations. To assist the implementation of these techniques for tuberculosis (TB) surveillance and outbreak investigations at European level there is a need for inter-country collaboration and standardization. This demands more information on molecular typing practices and capabilities of individual countries. We aimed to review the use of molecular/genomic typing for TB surveillance in European Union and European Economic Area countries in 2016; assess its public health value; and collect experiences on typing data use for cross-border cluster investigations.

Method

A web-based questionnaire was provided to all TB National Focal Points. The questionnaire consisted of three parts: i) Use and integration of molecular and genomic typing data into TB surveillance; ii) Cross-border cluster investigation and international collaboration, and iii) Perception and evaluation of public health benefits of molecular and genomic typing for TB surveillance.

Results

Of 26 responding countries, 20 used molecular typing for TB surveillance, including nine applying WGS. The level of integration into the national surveillance was heterogeneous. Among six countries not using typing for TB surveillance, more than half planned its implementation soon. Overall, most countries perceived an added public health value of molecular typing for TB control. Concerning international cluster investigations, countries had little experience and did not have standard protocols to exchange typing data.
Conclusion
Our study shows a wide use of molecular and genomic typing data for TB surveillance in EU/EEA countries and reveals that transition to WGS-based typing is ongoing or is considered in most countries. However, our results also show a high heterogeneity in the use and integration of typing data for TB surveillance. Standardization of typing data use for TB surveillance is needed and formal procedures should be developed to facilitate international collaboration.

Introduction
Molecular typing of *Mycobacterium tuberculosis* complex (MTB) is increasingly used to strengthen tuberculosis (TB) surveillance. 24-locus mycobacterial interspersed repetitive units variable number of tandem repeats (24 MIRU-VNTR) has become a standard tool [1, 2]. Yet, the transition to whole genome sequencing (WGS) is ongoing in the European Union (EU) and European and Economic Area (EEA) [3, 4]. Due to its higher discriminatory power [5] and potential to detect drug resistance [6], WGS is becoming a powerful tool to investigate TB outbreaks [7–10]. Recently, national TB contact points and reference laboratories supported by the European Centre for Disease Prevention and Control (ECDC) have used WGS-based typing to detect and clarify cross-border TB transmission [11, 12].

A recent review of the European Reference Laboratory Network for Tuberculosis (ERLTB-Net) [5], however, underlines that the appropriate role of WGS in TB surveillance remains to be defined and further evidence on the technical capacity across EU/EEA is needed before WGS-based surveillance for multidrug-resistant (MDR) TB can be operationalized [13, 14]. We have performed a questionnaire survey among EU/EEA Member States to i) review current practices in application of molecular/genomic typing for TB surveillance and capacity of transition to WGS-based typing; ii) explore the capability to use the typing in cross-border cluster investigations; and iii) assess its added public health value for TB surveillance, and to identify areas for future actions.

Methods
A web-based questionnaire (Acuity 4 Survey, Voxco) was developed. It was piloted amongst five volunteering countries (Denmark, Italy, the Netherlands, Norway and Sweden) and thereafter adjusted.

The survey was conducted between September and November 2016 among TB National Focal Points of all EU/EEA Member States. Participants were encouraged to consult with other competent bodies in their country if needed. Countries not responding were followed up with two reminder emails.

The questionnaire consisted of three parts: i) Use and integration of molecular/genomic typing data into TB surveillance; ii) Cross-border cluster investigation and international collaboration, and iii) Perception and evaluation of public health benefits. The questionnaire comprised 23 closed- and three open-ended questions. Data protection was guaranteed by the Server architecture and the data protection concept of the RKI and approved by the data protection and legal departments of the RKI, resulting in a waiver for ethical review.

We performed a descriptive analysis of the collected data using Stata 14.0. Maps were generated using Regiograph (http://regiograph.gfk.com/).
Results

Of the twenty-six responding EU/EEA countries (26/31; 84% response rate), 20 countries did and six did not use molecular/genomic typing for TB surveillance (Fig 1).

Countries using molecular/genomic typing for TB surveillance

Of the 20 countries that used typing data for surveillance, 19 used them for national surveillance and four also at sub-national level. Spain used typing data only at sub-national level (Table 1). 24 MIRU-VNTR was used by all countries. Seven countries used exclusively 24 MIRU-VNTR and 13 combined it with either spoligotyping (4/20); WGS (5/20); spoligotyping and WGS (3/20) or IS6110-RFLP, spoligotyping and WGS (1/20). Overall, nine countries (Austria, Denmark, Finland, France, Italy, Norway, Spain, Sweden and UK) used WGS, ten more countries considered its introduction (Table 1).

Of WGS-using countries, Austria used WGS as primary typing method (1/9) and five countries (Denmark, Finland, Italy, Norway and Spain) as secondary typing method to improve the resolution within spoligotyping and/or 24 MIRU-VNTR clusters (Table 2). Sweden was planning to use WGS as primary typing method in September 2016, and England (unknown for Wales, Northern Ireland & Scotland), Norway and Denmark from 2017. France was implementing progressively the use of WGS as high-resolution typing tool and Italy also used WGS for determination of drug-resistance.

All 20 countries typed multidrug and extensively drug-resistant (M/XDR) TB isolates using 24 MIRU-VNTR. Average estimated typing coverage in 2015 was 95% (range 53–100). All but three countries (Bulgaria, Estonia and Germany) also systematically typed outbreak isolates and 13 countries typed all kinds of MTB isolates, eleven with a coverage of ≥ 90%.
| Country       | Molecular typing surveillance (since year) | Administrative level | Use | Method used for surveillance | Molecular typing for surveillance (since year) | Method outbreak investigations | Typing laboratory* | Median reporting time (in days) | Kind of isolates | Coverage in 2015 (%) | Case-based integration | Method outbreak investigation | MTB isolates | M/XDR isolates | Coverage in 2015 (%) | Case-based integration | Method outbreak investigation | MTB isolates | M/XDR isolates | Coverage in 2015 (%) | Case-based integration | Method outbreak investigation |
|---------------|------------------------------------------|----------------------|-----|------------------------------|-----------------------------------------------|-------------------------------|----------------------|------------------------------|----------------|----------------|----------------------|--------------------------------|--------------|----------------|----------------|----------------------|--------------------------------|--------------|----------------|----------------|----------------------|--------------------------------|
| Austria       | Yes (2002)                               | National             | Yes | Spoligo, 24 MIRU-VNTR, WGS, MDR-MTB | No                                            | No                            | Spoligo, 24 MIRU-VNTR | 2017         | 100            | MTB 100             | Yes, national                   | NA            | 30                | 30            | No                   | Spoligo, 24 MIRU-VNTR, WGS, MDR-MTB | 10 | 30 | 30 | No | Spoligo, 24 MIRU-VNTR, WGS, MDR-MTB |
| Belgium       | Yes (2000)                               | National             | No  | Yes                           | Yes                                           | No                            | Spoligo, 24 MIRU-VNTR | 2000         | 100            | MTB 90              | Yes, national                   | 250           | 30                | 30            | Yes, national | Spoligo, 24 MIRU-VNTR | NA            | 30 | 30 | Yes, national | Spoligo, 24 MIRU-VNTR |
| Bulgaria      | Yes (2007)                               | National             | Yes | MIRU-VNTR                     | No                                            | No                            | MIRU-VNTR             | 2007         | 100            | MTB 97              | Yes, national                   | 100           | 300               | 300           | No                   | MIRU-VNTR                                      | 97 | 30 | 97 | No | MIRU-VNTR |
| Czech Republic| Yes (2007)                               | National             | Yes | MIRU-VNTR                     | Yes                                           | No                            | MIRU-VNTR             | 2007         | 100            | MTB 95              | Yes, national                   | 100           | 100               | 100           | Yes, national | MIRU-VNTR                                      | 95 | 100 | 95 | Yes, national | MIRU-VNTR |
| Denmark       | Yes (2007)                               | National             | No  | Yes                           | Yes                                           | No                            | MIRU-VNTR             | 2007         | 100            | MTB 95              | Yes, national                   | 100           | 100               | 100           | Yes, national | MIRU-VNTR                                      | 95 | 100 | 95 | Yes, national | MIRU-VNTR |
| Estonia       | Yes (2003)                               | National             | Yes | MIRU-VNTR                     | Yes                                           | No                            | MIRU-VNTR             | 2003         | 100            | MTB 97              | Yes, national                   | 100           | 100               | 100           | Yes, national | MIRU-VNTR                                      | 97 | 30 | 97 | No | MIRU-VNTR |
| Finland       | Yes (2000)                               | National             | Yes | MIRU-VNTR                     | Yes                                           | No                            | MIRU-VNTR             | 2000         | 100            | MTB 100             | Yes, national                   | 100           | 100               | 100           | Yes, national | MIRU-VNTR                                      | 100 | 100 | 100 | Yes, national | MIRU-VNTR |
| France        | Yes (1995)                               | National             | No  | MIRU-VNTR                     | Yes                                           | No                            | MIRU-VNTR             | 1995         | 100            | MTB 95              | Yes, national                   | 100           | 100               | 100           | Yes, national | MIRU-VNTR                                      | 95 | 100 | 95 | Yes, national | MIRU-VNTR |
| Germany       | Yes (1992)                               | National             | Yes | MIRU-VNTR                     | Yes                                           | No                            | MIRU-VNTR             | 1992         | 100            | MTB 95              | Yes, national                   | 100           | 100               | 100           | Yes, national | MIRU-VNTR                                      | 95 | 100 | 95 | Yes, national | MIRU-VNTR |
| Hungary       | Yes (2001)                               | National             | Yes | MIRU-VNTR                     | Yes                                           | No                            | MIRU-VNTR             | 2001         | 100            | MTB 95              | Yes, national                   | 100           | 100               | 100           | Yes, national | MIRU-VNTR                                      | 95 | 100 | 95 | Yes, national | MIRU-VNTR |
| Ireland       | Yes (2011)                               | National, local      | Yes | MIRU-VNTR                     | Yes                                           | No                            | MIRU-VNTR             | 2011         | 100            | MTB 95              | Yes, national                   | 100           | 100               | 100           | Yes, national | MIRU-VNTR                                      | 95 | 100 | 95 | Yes, national | MIRU-VNTR |
| Italy         | Yes (2004)                               | National, regional, local | Yes | MIRU-VNTR                     | Yes                                           | No                            | MIRU-VNTR             | 2004         | 100            | MTB 95              | Yes, regional, local             | 100           | 100               | 100           | Yes, regional, local | MIRU-VNTR                                      | 95 | 100 | 95 | Yes, regional, local | MIRU-VNTR |
| Latvia        | No (Planned for 2011)                    | National             | Yes | MIRU-VNTR                     | Yes                                           | No                            | MIRU-VNTR             | 2011         | 100            | MTB 95              | Yes, national                   | 100           | 100               | 100           | Yes, national | MIRU-VNTR                                      | 95 | 100 | 95 | Yes, national | MIRU-VNTR |
| Lithuania     | No (Planned for 2012)                    | National             | Yes | MIRU-VNTR                     | Yes                                           | No                            | MIRU-VNTR             | 2012         | 100            | MTB 95              | Yes, national                   | 100           | 100               | 100           | Yes, national | MIRU-VNTR                                      | 95 | 100 | 95 | Yes, national | MIRU-VNTR |
| Luxembourg    | No (Planned for 2016)                    | National             | Yes | MIRU-VNTR                     | Yes                                           | No                            | MIRU-VNTR             | 2016         | 100            | MTB 95              | Yes, national                   | 100           | 100               | 100           | Yes, national | MIRU-VNTR                                      | 95 | 100 | 95 | Yes, national | MIRU-VNTR |
| Malta         | No (Planned for 2017)                    | National             | Yes | MIRU-VNTR                     | Yes                                           | No                            | MIRU-VNTR             | 2017         | 100            | MTB 95              | Yes, national                   | 100           | 100               | 100           | Yes, national | MIRU-VNTR                                      | 95 | 100 | 95 | Yes, national | MIRU-VNTR |
| Netherlands   | Yes (1993)                               | National             | Yes | MIRU-VNTR                     | Yes                                           | No                            | MIRU-VNTR             | 1993         | 100            | MTB 95              | Yes, national                   | 100           | 100               | 100           | Yes, national | MIRU-VNTR                                      | 95 | 100 | 95 | Yes, national | MIRU-VNTR |
| Norway        | Yes (2006)                               | National             | Yes | MIRU-VNTR                     | Yes                                           | No                            | MIRU-VNTR             | 2006         | 100            | MTB 95              | Yes, national                   | 100           | 100               | 100           | Yes, national | MIRU-VNTR                                      | 95 | 100 | 95 | Yes, national | MIRU-VNTR |
| Poland        | Yes (2016)                               | National             | Yes | MIRU-VNTR                     | Yes                                           | No                            | MIRU-VNTR             | 2016         | 100            | MTB 95              | Yes, national                   | 100           | 100               | 100           | Yes, national | MIRU-VNTR                                      | 95 | 100 | 95 | Yes, national | MIRU-VNTR |
| Portugal      | Yes (2014)                               | National             | No  | MIRU-VNTR                     | No                                            | No                            | MIRU-VNTR             | 2014         | 100            | MTB 95              | Yes, national                   | 100           | 100               | 100           | Yes, national | MIRU-VNTR                                      | 95 | 100 | 95 | Yes, national | MIRU-VNTR |
| Romania       | No (Planned for 2019)                    | National             | Yes | MIRU-VNTR                     | Yes                                           | No                            | MIRU-VNTR             | 2019         | 100            | MTB 95              | Yes, national                   | 100           | 100               | 100           | Yes, national | MIRU-VNTR                                      | 95 | 100 | 95 | Yes, national | MIRU-VNTR |

*Typing laboratory*: spoligo, WGS, MIRU-VNTR, MDR-MTB, MTB, M/XDR-MTB; MTB isolates: MTB, M/XDR-MTB, polyresistant strains; Coverage in 2015 (%): MTB 90 100, NRL 30 60; Method outbreak investigation: MTB-MTB outbreak (in some regions all MTB isolates), MDR-MTB outbreak (in some regions all MDR-MTB isolates).
| Country       | Molecular typing for surveillance (since year) | Administrative level | Methods used                                      | WGS-based typing Use (if no) Planned, year | Kind of isolates typed | Coverage in 2015 (%) | Typing laboratory | Median reporting time (in days) | Case-based integration | Method outbreak investigations |
|--------------|-----------------------------------------------|----------------------|--------------------------------------------------|---------------------------------------------|------------------------|----------------------|---------------------|-------------------------------|----------------------|-------------------------------|
| Slovenia     | Yes (2000)                                    | National             | 24 MIRU-VNTR                                     | MTB isolates | M/XDR isolates | spoligo 24 MIRU-VNTR | 100 | 100 | NRL | 7 | Yes, national | 24 MIRU-VNTR |
| Spain        | Yes                                           | Regional, local      | IS6110-RFLP; spoligo; 12 MIRU-VNTR; 24 MIRU-VNTR; WGS | MTB isolates | M/XDR-MTB outbreak | Not known | Not known | NRL; PLL | Not known | Yes, regional, local | Is6110-RFLP; spoligo; 12 MIRU-VNTR; 24 MIRU-VNTR; WGS |
| Sweden       | Yes (1996)                                    | National             | spoligo; 24 MIRU-VNTR; WGS                       | MTB isolates | M/XDR-MTB outbreak | Not known | Not known | NRL; clin. lab. | 14 | 14 | Yes, national | Is6110-RFLP; spoligo; 12 MIRU-VNTR; 24 MIRU-VNTR; WGS |
| United Kingdom | Yes (2010)                                  | National             | 24 MIRU-VNTR; WGS                                | MTB isolates | M/XDR-MTB outbreak | Not known | Not known | NRL; RRL | 65 | Yes, national, regional, local | 24 MIRU-VNTR; WGS |

* Information in this table refers to spoligo- and 24 MIRU-VNTR. Information exclusively on WGS is provided in Table 2. Pink denotes countries not using molecular typing for TB surveillance, and therefore the questions are not applicable.

1 At the national level and for M/XDR-MTB isolates.

2 It is planned that 24 MIRU-VNTR will be simultaneously used.

Clin. Lab.: clinical laboratories; Com. typ. serv.: commercial typing service; MDR: multidrug-resistant tuberculosis; MIRU-VNTR: Mycobacterial Interspersed Repetitive Units - Variable Number of Tandem Repeat; MTB: Mycobacterium tuberculosis; NA: not applicable; NRL: National Reference Laboratory; PFG: pulsed-field gel electrophoresis; PLL: peripheral level laboratories; rep-PCR: repetitive sequence-based-PCR; RFLP: Restriction fragment length polymorphism; RRL: regional reference laboratory; WGS: whole genome sequencing; XDR: extensively drug-resistant tuberculosis.

https://doi.org/10.1371/journal.pone.0210080.t001
WGS was used to type all MTB isolates in two countries (Denmark and Sweden); both M/XDR and outbreak isolates in four countries (Austria, Finland, Italy and Spain); only M/XDR isolates in France and only outbreak isolates in Norway. The UK was in transition from 24 MIRU-VNTR to WGS for all MTB isolates (Table 1). Three countries (Denmark, Finland and Sweden) typed >90% of their M/XDR isolates using WGS.

Where is typing performed? All 20 countries had a National Reference Laboratory (NRL) that performed molecular/genomic typing. Additional typing laboratories included regional reference laboratories (2/20); peripheral level laboratories (2/20); clinical laboratories (2/20); research institutes (1/20) and a commercial typing service provider inside the country (1/20) or outside the country (1/20). All countries using WGS-based typing performed it in their NRL. In France and Spain, WGS-based typing was also performed by a commercial typing service provider and peripheral level laboratories, respectively.

The estimated median timespan between a MTB positive culture and reception of the typing results by surveillance units was 30 days for both 24 MIRU-VNTR (interquartile range, IQR, 14–60) and WGS (IQR 14–40).

Who is analyzing typing data? Analysis of 24 MIRU-VNTR data to identify molecular clusters was performed by the typing laboratory (11/20); jointly by typing laboratory and surveillance units (7/20) or entirely by the surveillance unit (2/20).

Analysis of WGS data was mainly performed by the typing laboratory in Austria, Denmark, Finland, Italy and Norway, and jointly by typing laboratory and surveillance unit in France and Sweden, and by the TB surveillance unit in Spain. In England the analytical pipeline setup was in progress.

Integration of typing data into TB notification databases. Sixteen of 20 countries integrated the molecular/genomic typing data into the TB notification database on a case-based level. This integration occurred mostly at national level (14/16) and in Ireland and UK also...
sub-nationally. In Italy and Spain, integration only occurred sub-nationally. Only Italy and Spain integrated WGS results into a notification database (sub-nationally). Three countries (Czech Republic, Germany and Portugal) did not systematically integrate them, one country replied “unknown”.

Countries not using molecular/genomic typing for TB surveillance

Six countries (Hungary, Latvia, Lithuania, Luxembourg, Malta and Romania) did not use molecular typing data for TB surveillance (Table 3). However, four of them considered its implementation soon (Hungary, Lithuania, Luxembourg and Romania) and three of them (Lithuania, Luxembourg and Romania) considered using WGS. Three countries (Hungary, Latvia and Lithuania) had performed molecular typing (mostly 24 MIRU-VNTR) of MTB isolates for research (3/6) or laboratory cross-contamination investigation (1/6).

Barriers for using molecular/genomic typing data in TB surveillance

Most countries (18/26) identified barriers for using typing data in TB surveillance (Table 4). “Financial constraints” was the most common barrier; both among countries using typing (10/20) and among countries not using it (5/6). Besides “financial constraints”, “human resources” (8/20) was most frequently mentioned by countries using molecular typing, while “data management and analysis” (3/6) by countries not using typing.

As to specific barriers to WGS-based typing (Table 5), mainly “financial constraints” were reported, (6/9 countries performing WGS and 11/17 countries not performing WGS). Countries using WGS also highlighted “human resources” (5/9) as significant barrier. Countries not using WGS underlined “data management and analysis” (10/17) as relevant barrier. Six countries (including countries using and not using WGS) did not perceive WGS-specific barriers.

Most countries claimed that standardization of WGS data analysis and outbreak investigation should be improved and that collaboration and data sharing should be facilitated. Several countries mentioned that countries with WGS capacity could support other countries without capacity.

Table 3. Overview of M. tuberculosis molecular typing practices in countries that do not use molecular typing for TB surveillance in European Union/European Economic Area countries.

| Country    | Molecular typing use                                      | Molecular typing methods | Plan for molecular typing for TB surveillance | WGS plan as typing method? |
|------------|----------------------------------------------------------|--------------------------|---------------------------------------------|----------------------------|
| Hungary    | Research; Laboratory cross contamination investigation   | 24 MIRU-VNTR             | Yes                                        | No                         |
| Latvia     | Research IS6110-RFLP; spoligo                            | IS6110-RFLP              | Not known                                  | NA                         |
| Lithuania  | Research                                                | 24 loci MIRU-VNTR        | Yes                                        | Yes                        |
| Luxembourg | No                                                      | None                     | Yes                                        | Yes                        |
| Malta      | No                                                      | None                     | No                                         | NA                         |
| Romania    | No                                                      | None                     | Yes                                        | Yes                        |

MIRU-VNTR: Mycobacterial Interspersed Repetitive Units - Variable Number of Tandem Repeat; NA: not applicable; NRL: National Reference Laboratory; PFGE: pulsed-field gel electrophoresis; PLL: peripheral level laboratories; rep-PCR: repetitive sequence-based-PCR; RFLP: Restriction fragment length polymorphism; RRL: regional reference laboratory; XDR: extensively drug-resistant tuberculosis; WGS: whole genome sequencing.

https://doi.org/10.1371/journal.pone.0210080.t003
Cross-border cluster investigation and international collaboration

Fourteen of the 26 responding countries had been contacted at least once by another EU/EEA Member State to participate in cross-border cluster investigations; only seven countries (Austria, Germany, Ireland, the Netherlands, Slovenia, Sweden and UK) had actively approached another EU/EEA country for international collaboration.

Six countries (Ireland, the Netherlands, Norway, Slovenia, Sweden and UK) had established standard operational procedures (SOPs) to perform national cluster investigations but none had a SOP for international investigations. Countries relied on the following legal basis for the international exchange of patient information in cross-border cluster investigation: Decision
No. 1082/2013/EU of the European Parliament and of the Council (8/26) [15], the International Health Regulation and Implementation Act of the country (8/26), and their respective national law (4/26). Half of the countries (13/26) did not know which legal framework applied.

Eight countries reported barriers for cross-border cluster investigations, seven reported that there were none, eleven did not know. Main barriers were: “different levels of integration of molecular typing data” (6/8); “[lack of] standardization of molecular typing methodologies” (5/8); “reluctance to share personal data of patients” (4/8) and “legal constraints” (4/8; Table 6).
Public health benefits

All countries perceived a public health benefit of using molecular/genomic typing for TB surveillance (Fig 2): 1. Detection of unknown transmission links (24/26, formally evaluated by 16 countries); 2. Improvement of contact investigation (24/26, formally evaluated by 13 countries); 3. Identification and investigation of high risk strains (23/26, formally evaluated by 14 countries) and 4. Detection of clusters across different regions (23/26, formally evaluated by 12 countries).

As to specific benefits of using WGS-based typing (Fig 2), 24/26 countries perceived a benefit, two countries did not know (Bulgaria and Malta). The main benefits were: 1. Higher
discriminatory power (19/26); 2. Improved contact investigation (16/26) and 3. Untie potential outbreaks (16/26). Of the countries using molecular/genomic typing, most (19/20) considered that using WGS-based typing was beneficial, compared to two third (4/6) of the countries that did not use typing. WGS-using countries mostly considered WGS useful because it provides additional information, namely drug resistance.

**Discussion**

Our survey shows that most EU/EEA Member States use molecular/genomic typing data for TB surveillance and the transition to WGS is ongoing. Our results also reveal substantial differences in the use and integration of typing data into national TB surveillance systems and identify financial constraints as the main barrier to a broader use, as well as limited experience in cross-border cluster investigation and a lack of respective SOPs. Most countries recognized a public health benefit of molecular typing and an additional benefit of using WGS-based typing.

The implementation of TB molecular surveillance is highly heterogeneous in EU/EEA countries in terms of the kind of typing laboratories, the selection of isolates, the coverage, the analytical approach used, and whether typing data are integrated into TB notification databases. In three countries typing results took ≥ 240 days to reach the surveillance units, which...
may limit the impact on TB control and is in contrast to the increasing speed of typing, e.g., in view of forthcoming genomic typing using direct samples [16].

Almost half of the responding countries that do not yet use molecular typing for TB surveillance, use it for other purposes such as diagnosis or research, and Hungary and Malta contribute to the ECDC MDR-TB molecular surveillance project while not using typing data for their national TB surveillance [17]. In Malta MDR-TB isolates are typed by the Dutch NRL [18] and in Hungary together by the Dutch and the Hungarian NRLs.

Overall, major barriers identified for using molecular typing data for TB surveillance were financial and human resource related. Countries using molecular typing also identified the “utilization of the data for TB control” as a barrier, e.g., clustering does not necessarily mean recent transmission [19–21] and therefore linking typing data to detailed epidemiological information of clustered cases is essential.

TB spread has an international dimension, as shown by the ECDC MDR-TB molecular surveillance project [17, 18]. WGS is being increasingly used to detect and clarify international outbreaks, such as an international MDR-TB cluster among asylum seekers that continues to expand across different EU countries [12], and a XDR-TB cross-border outbreak [22]. Rapid sharing of molecular/genomic typing data and epidemiological information between countries is important. Another investigation of a MDR-TB cluster in Austria, Romania and Germany using WGS [11] has demonstrated the need for establishing protocols for data sharing, which is supported by our results and previous studies [23]. However, EU/EEA countries still have limited experience on conducting cross-border cluster investigations and lack respective SOPs. This can potentially hamper international cluster investigations and subsequent measures of transmission control.

The major barriers for international collaboration were related to the different levels of typing data integration and insufficient standardization of molecular methodologies and data analysis. This may be even more complex when WGS-typing is used and exchanged given that data interpretation is more dependent on laboratory protocols and analysis pipelines [5, 24]. Therefore, standardized laboratory methodologies, analytical approaches and terminology are essential to ensure interchangeable among countries [25]. The finding that WGS-based typing is currently mostly exclusively performed by NRLs represents an opportunity for developing respective international standards before the laboratory network gets potentially more complex [14]. In 2017, ECDC initiated a pilot project on the use of WGS for molecular typing and characterization of M. tuberculosis in the EU and EEA. The project aims to standardize WGS laboratory procedures and bioinformatic analysis and to provide access to WGS for EU/EEA Member States that do not yet have capacity for WGS of M. tuberculosis [26].

None of the participating countries mentioned the quality and reliability of molecular/genomic typing results as a barrier to international collaboration. Proficiency testing of MIR-U-VNTR typing has shown that laboratories face challenges with the inter- and intra-laboratory reproducibility of results [27]. The 2016 ECDC facilitated external quality assessment for 24 MIRU-VNTR typing showed that four of the 16 participating laboratories did not reach the threshold level for certification (unpublished data), which can critically compromise cluster investigations.

Demonstrating the added public health benefit of integrating molecular/genomic typing into TB surveillance systems remains challenging. [5] even though multiple scientific studies emphasize the power of molecular typing to clarify TB outbreaks [28, 29] particularly using WGS [7–10, 30]; monitor within country domestic transmission [31–33]; or identify high risk strains [34]. In our survey, surveillance units recognize the benefit of typing data for TB surveillance, especially for the detection of unknown transmission links and improvement of TB outbreak and contact investigations.
Strikingly, the countries not using molecular typing in-country mostly have a high percentage of MDR-TB cases [35]. And in several documented incidents, molecular typing performed abroad pointed M/XDR-TB transmission scenarios in these countries [11, 12]. This places even higher importance on timely international collaboration and information exchange, as well as on integrating these countries in molecular typing programs.

Previous economic evaluations of integrated molecular surveillance systems in England and the Netherlands suggested that the contribution of molecular typing to improve contact investigations is limited and the system was not cost-effective in the investigation period [36–38]. A recent study has shown the limited power of MIRU-VNTR to predict MTB genomic relatedness [39] but expects that the introduction of WGS-based typing may change this picture, given its higher discriminatory power and drug resistance detection [6, 40]. Further formal evaluations specifying and comparing different typing methods are thus needed, since different typing methods lead to different conclusions and demand different resources [40]. The area-wide introduction of a routine WGS-service by Public Health England offers a special opportunity to evaluate the added value and the costs of WGS-based typing for public health.

Limitations
Our EU/EEA survey provides a general overview but may not replace in-depth technical exchange on integrated molecular TB surveillance systems. Despite the high response rate (84%), selection bias may not be entirely excluded, and our results may overestimate the current use of typing for TB surveillance in Europe. Since this is a rapid evolving field, some countries might have advanced in the implementation of WGS-based typing since the survey was performed, e.g., in the UK.

Conclusions
Our study shows a wide use of molecular/genomic typing data for TB surveillance in EU/EEA countries and an ongoing transition to WGS-based typing. A high heterogeneity in their use and integration stress the need for timely standardization of WGS-based typing procedures and exchange of results, as well as administrative and legal frameworks and SOPs to facilitate international collaboration. The knowledge of pioneer countries and the perceived and observed public health benefits of molecular typing for TB control area favourable premise to tackle remaining challenges.

Acknowledgments
We would like to thank our colleagues from the Robert Koch Institute, the European Centre for Disease Prevention and Control and the German National Reference Laboratory for their helpful feedback on the draft questionnaire. Funding for this study was provided by Special research funds for molecular Surveillance of the Robert Koch Institute.

Members of the Survey study group: Austria: A. Indra; Belgium: M. Wanlin; Bulgaria: E. Bachiyska; Croatia: A. Jurcev-Savicevic; Czech Republic: I. Zemanova; Denmark: T. Lillebaek; Estonia: P. Viiklepp; Finland: M. Haanperä; France: W. Sougakoff; Hungary: A. Bakos; Ireland: S. Jackson; Italy: D. M. Cirillo; Latvia: I. Norvaisa; Lithuania: E. Vasiliauskiene; Luxembourg: P. Reichert; Malta: P. Caruana; Netherlands: G. de Vries; Norway: K. Ronning; Poland: E. Augustynowicz-Kopeć; Portugal: R. Duarte; Romania: D. Homorodean; Slovenia: P. Svetina; Spain: L. Sánchez-Cambronero; Sweden: R. Groenheit; United Kingdom: J. A. Davidson.
Author Contributions

Conceptualization: Marta Andrés, Marieke J. van der Werf, Csaba Ködmön, Walter Haas, Lena Fiebig.

Data curation: Stefan Albrecht.

Formal analysis: Marta Andrés, Stefan Albrecht.

Investigation: Marta Andrés.

Methodology: Marta Andrés, Marieke J. van der Werf, Csaba Ködmön, Lena Fiebig.

Project administration: Marta Andrés.

Software: Stefan Albrecht.

Supervision: Walter Haas, Lena Fiebig.

Validation: Marta Andrés.

Visualization: Marta Andrés.

Writing – original draft: Marta Andrés, Lena Fiebig.

Writing – review & editing: Marieke J. van der Werf, Csaba Ködmön, Stefan Albrecht, Walter Haas.

References

1. de Beer JL, Kremer K, Kodmon C, Supply P, van Soolingen D. First worldwide proficiency study on variable-number tandem-repeat typing of Mycobacterium tuberculosis complex strains. J Clin Microbiol. 2012; 50(3):662–9. https://doi.org/10.1128/JCM.00607-11 PMID: 22170917

2. Supply P, Allix C, Lesjean S, Cardoso-Oelermann M, Rusch-Gerdes S, Willery E, et al. Proposal for standardization of optimized mycobacterial interspersed repetitive unit-variable-number tandem repeat typing of Mycobacterium tuberculosis. Journal of Clinical Microbiology. 2006; 44(12):4498–510. https://doi.org/10.1128/JCM.01392-06 PMID: 17005759

3. Struelens MJ, Brisse S. From molecular to genomic epidemiology: transforming surveillance and control of infectious diseases. Euro Surveill. 2013; 18(4):20386. PMID: 23369387

4. Walker TM, Cruz ALG, Peto TE, Smith EG, Esmail H, Crook DW. Tuberculosis is changing. Lancet Infect Dis. 2017; 17(4):359–61. https://doi.org/10.1016/S1473-3099(17)30123-8 PMID: 28298254

5. Nikolayevskyy V, Kranzer K, Niemann S, Drobniewski F. Whole genome sequencing of Mycobacterium tuberculosis for detection of recent transmission and tracing outbreaks: A systematic review. Tuberculosis. 2016; 98:77–85. https://doi.org/10.1016/j.tube.2016.02.009 PMID: 27156621

6. Papaventisis D, Casali N, Kontsevaya I, Drobniewski F, Cirillo DM, Nikolayevskyy V. Whole genome sequencing of Mycobacterium tuberculosis for detection of drug resistance: a systematic review. Clin Microbiol Infect. 2016.

7. Roetzer A, Diel R, Kohl TA, Ruckert C, Nubel U, Blom J, et al. Whole genome sequencing versus traditional genotyping for investigation of a Mycobacterium tuberculosis outbreak: a longitudinal molecular epidemiological study. PLoS Medicine. 2013; 10(2):e1001387. https://doi.org/10.1371/journal.pmed.1001387 PMID: 23424287

8. Casali N, Broda A, Harris SR, Parkhill J, Brown T, Drobniewski F. Whole Genome Sequence Analysis of a Large Isoniazid-Resistant Tuberculosis Outbreak in London: A Retrospective Observational Study. PLoS Med. 2016; 13(10):e1002137. https://doi.org/10.1371/journal.pmed.1002137 PMID: 27701423

9. Satta G, Witney AA, Shorten RJ, Karlkowska M, Lipman M, McHugh TD. Genetic variation in Mycobacterium tuberculosis isolates from a London outbreak associated with isoniazid resistance. BMC Med. 2016; 14(1):117. https://doi.org/10.1186/s12916-016-0659-6 PMID: 27530812

10. Walker TM, Ip CL, Harrell RH, Evans JT, Kapatai G, Dedicoat MJ, et al. Whole-genome sequencing to delineate Mycobacterium tuberculosis outbreaks: a retrospective observational study. The Lancet Infectious Diseases. 2013; 13(2):137–46. https://doi.org/10.1016/S1473-3099(12)70277-3 PMID: 23158499
11. Fiebig L, Kohl TA, Popovici O, Mühlenfeld M, Indra A, Homorodean D, et al. A joint cross-border investigation of a cluster of multidrug-resistant tuberculosis in Austria, Romania and Germany in 2014 using classic, genotyping and whole genome sequencing methods: lessons learnt. Euro Surveill. 2017; 22(2).

12. European Centre for Disease Prevention and Control (ECDC). Multidrug-resistant tuberculosis in migrants, multicountry cluster– 13 April 2017. Stockholm: ECDC; 2017. Available from: https://ecdc.europa.eu/en/publications/Publications/RRA-xdr-tuberculosis-romania-october-2016.pdf. (last accessed on 20 December 2018).

13. European Centre for Disease Prevention and Control (ECDC). ECDC roadmap for integration of molecular and genomic typing into European-level surveillance and epidemic preparedness–Version 2.1, 2016–19. Stockholm: ECDC; 2016. Available from: http://ecdc.europa.eu/en/publications/Publications/molecular-typing-EU-surveillance-epidemic-preparedness-2016-19-roadmap.pdf. (last accessed on 20 December 2018).

14. European Centre for Disease Prevention and Control (ECDC). Expert opinion on whole genome sequencing for public health surveillance. Stockholm: ECDC; 2016. Available from: https://ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/whole-genome-sequencing-for-public-health-surveillance.pdf. (last accessed on 20 December 2018).

15. van Belkum A, Tassios PT, Dijkshoorn L, Haeggman S, Cookson B, Fry NK, et al. Guidelines for the validation and application of typing methods for use in bacterial epidemiology. Clin Microbiol Infect. 2007; 13 Suppl 3:1–46.

17. European Centre for Disease Prevention and Control (ECDC). Molecular typing for surveillance of multidrug-resistant tuberculosis in the EU/EEA–March 2017. Stockholm: ECDC; 2017. Available from: http://ecdc.europa.eu/en/publications/Publications/multidrug-resistant-tuberculosis-molecular-typing-surveillance.pdf. (last accessed on 20 December 2018).

18. De Beer JL, Kodmon C, van der Werf MJ, van Ingen J, van Soolingen D. Molecular surveillance of multi- and extensively drug-resistant tuberculosis transmission in the European Union from 2003 to 2011. Euro Surveill. 2014; 19(11).

19. de Vries G, Baars H, Sebek M, van Hest N, Richards JDH. Transmission classification model to determine place and time of infection of tuberculosis cases in an urban area. J Clin Microbiol. 2008; 46 (12):3924–30. https://doi.org/10.1128/JCM.00793-08 PMID: 18842933

20. Braden CR, Templeton GL, Cave MD, Valway S, Onorato IM, Castro KG, et al. Interpretation of restriction fragment length polymorphism analysis of Mycobacterium tuberculosis isolates from a state with a large rural population. J Infect Dis. 1997; 175(6):1446–52. PMID: 9180185

21. Glynn J, Vyonycky E, Fine P. Influence of sampling on estimates of clustering and recent transmission of Mycobacterium tuberculosis derived from DNA fingerprinting techniques. Am J Epidemiol. 1999; 149 (4):366–71. PMID: 10025480

22. European Centre for Disease Prevention and Control (ECDC). Extensively drug-resistant (XDR) tuberculosis–multi-country cluster, Romania. 21 October 2016. Stockholm: ECDC; 2016. Available from: https://ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/RRA-xdr-tuberculosis-romania-october-2016.pdf. (last accessed on 20 December 2018).

23. Dara M, de Colombani P, Petrova-Benedict R, Centis R, Zellweger JP, Sandgren A, et al. Minimum package for cross-border TB control and care in the WHO European region: a Wolfheze consensus statement. Eur Respir J. 2012; 40(5):1081–90. https://doi.org/10.1183/09031936.00053012 PMID: 22653772

24. Kohl TA, Diel R, Harmsen D, Rothganger J, Walter KM, Merker M, et al. Whole-genome-based Mycobacterium tuberculosis surveillance: a standardized, portable, and expandable approach. Journal of clinical microbiology. 2014; 52(7):2479–86. https://doi.org/10.1128/JCM.00567-14 PMID: 24789177

25. Sabat AJ, Budimir A, Nashev D, Sa-Leao R, van Dijl J, Laurent F, et al. Overview of molecular typing methods for outbreak detection and epidemiological surveillance. Euro Surveill. 2013; 18(4):20380. PMID: 23369389

26. Tagliani E, Cirillo DM, Ködmön C, van der Werf MJ; EUSeqMyTB Consortium. EUSeqMyTB to set standards and build capacity for whole genome sequencing for tuberculosis in the EU. Lancet Infect Dis. 2018 Apr; 18(4):377.

27. de Beer JL, Kodmon C, van Ingen J, Supply P, van Soolingen D. Second worldwide proficiency study on variable number of tandem repeats typing of Mycobacterium tuberculosis complex. Int J Tuberc Lung Dis. 2014; 18(5):594–600. https://doi.org/10.5588/ijtld.13.0531 PMID: 24903798
28. Lillebaek T, Andersen AB, Rasmussen EM, Kamper-Jorgensen Z, Pedersen MK, Bjorn-Mortensen K, et al. Mycobacterium tuberculosis outbreak strain of Danish origin spreading at worrying rates among greenland-born persons in Denmark and Greenland. J Clin Microbiol. 2013; 51(12):4040–4. https://doi.org/10.1128/JCM.01916-13 PMID: 24068008

29. Macaraig M, Agerton T, Driver CR, Munsiff SS, Abdelwahab J, Park J, et al. Strain-specific differences in two large Mycobacterium tuberculosis genotype clusters in isolates collected from homeless patients in New York City from 2001 to 2004. J Clin Microbiol. 2006; 44(8):2890–6. https://doi.org/10.1128/JCM.00160-06 PMID: 16891508

30. Gardy JL, Johnston JC, Ho Sui SJ, Cook VJ, Shah L, Brodkin E, et al. Whole-genome sequencing and social-network analysis of a tuberculosis outbreak. N Engl J Med. 2011; 364(8):730–9. https://doi.org/10.1056/NEJMoa1003176 PMID: 21345102

31. Smit PW, Haaapera M, Rantala P, Couvin D, Lyytikainen O, Rastogi N, et al. Molecular epidemiology of tuberculosis in Finland, 2008–2011. PLoS one. 2013; 8(12):e85027. https://doi.org/10.1371/journal.pone.0085027 PMID: 24386443

32. Bidovec-Stojkovic U, Zolnir-Dovc M, Supply P. One year nationwide evaluation of 24-locus MIRU-VNTR genotyping on Slovenian Mycobacterium tuberculosis isolates. Respiratory medicine. 2011; 105 Suppl 1:S67–73.

33. Jonsson J, Hoffner S, Berggren I, Bruchfeld J, Ghebremichael S, Pennhag A, et al. Comparison between RFLP and MIRU-VNTR genotyping of Mycobacterium tuberculosis strains isolated in Stockholm 2009 to 2011. PLoS one. 2014; 9(4):e95159. https://doi.org/10.1371/journal.pone.0095159 PMID: 24733167

34. Shah NS, Auld SC, Brust JC, Mathema B, Ismail N, Moodley P, et al. Transmission of Extensively Drug-Resistant Tuberculosis in South Africa. N Engl J Med. 2017; 376(3):243–53. https://doi.org/10.1056/NEJMo1604544 PMID: 28099825

35. European Centre for Disease Prevention and Control (ECDC). Tuberculosis surveillance and monitoring in Europe, 2018. Stockholm: ECDC; 2018. Available from: https://ecdc.europa.eu/en/publications-data/tuberculosis-surveillance-and-monitoring-europe-2018. (last accessed on 20 December 2018).

36. Mears J, Vynnycky E, Lord J, Borgdorff MW, Cohen T, Crisp D, et al. The prospective evaluation of the TB strain typing service in England: a mixed methods study. Thorax. 2015.

37. Mears J, Abubakar I, Crisp D, Maguire H, Innes JA, Lilley M, et al. Prospective evaluation of a complex public health intervention: lessons from an initial and follow-up cross-sectional survey of the tuberculosis strain typing service in England. BMC public health. 2014; 14:1023. https://doi.org/10.1186/1471-2431-14-1023 PMID: 25273511

38. Lambregts-van Wezenbeek CS, Sebek MM, van Gerven PJ, de Vries G, Verver S, Kalisvaart NA, et al. Tuberculosis contact investigation and DNA fingerprint surveillance in The Netherlands: 6 years’ experience with nation-wide cluster feedback and cluster monitoring. The international journal of tuberculosis and lung disease: the official journal of the International Union against Tuberculosis and Lung Disease. 2003; 7(12 Suppl 3):S463–70.

39. Wyllie DH, Davidson JA, Grace Smith E, Rathod P, Crook DW, Peto TEA et al. A Quantitative Evaluation of MIRU-VNTR Typing Against Whole-Genome Sequencing for Identifying Mycobacterium tuberculosis Transmission: A Prospective Observational Cohort Study. EBioMedicine. 2018 Aug; 34:122–130. https://doi.org/10.1016/j.ebiom.2018.07.019 PMID: 30077721

40. Pankhurst LJ, Del Ojo Elias C, Votintseva AA, Walker TM, Cole K, Davies J, et al. Rapid, comprehensive, and affordable mycobacterial diagnosis with whole-genome sequencing: a prospective study. The Lancet Respiratory medicine. 2016; 4(1):49–58. https://doi.org/10.1016/S2213-2600(15)00466-X PMID: 26669883