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ABSTRACT

Background: Denmark is a low-incidence country for tuberculosis (TB) and multidrug-resistant (MDR) TB at 5 and 0.05 cases per 100,000 population, respectively. Until 2018, the transmission of MDR-TB was nonexistent except for a few pairwise related family cases. In this study, we describe the first MDR-TB outbreak in Denmark.

Methods: On the basis of genotyping of all *Mycobacterium tuberculosis* (Mtb) culture-positive cases in Denmark spanning 3 decades, 6 molecular- and epidemiologically linked Danish-born cases were identified as the first cluster of an MDR-TB in Denmark. The primary case was diagnosed posthumously in 2010 followed by 5 epidemiologically linked cases from 2018 to 2019.

Results and conclusion: Through a combination of routine Mtb genotyping and clinical epidemiological surveillance data, we identified the first Danish MDR-TB outbreak spanning 10 years and were able to disclose the specific transmission pathways in detail, which helped guide the outbreak investigations. The occurrence of an MDR-TB outbreak in a resource-rich low TB incidence setting such as Denmark highlights the importance of a collaborative control system combining classic contact tracing; timely identification of drug-resistant TB through rapid diagnostics; and a close collaboration between clinicians and classical- and molecular epidemiologists for the benefit of TB control.

Introduction

Until the recent advent of COVID-19 (Project HOPE, 2020), *Mycobacterium tuberculosis* (Mtb) remained the leading infectious disease that caused death worldwide, with approximately 10 million new cases and 1.3 million deaths per year (World Health Organization, 2020a). In contrast to the steady overall decrease in global tuberculosis (TB) incidence, the incidence of multidrug-resistant TB(MDR-TB) increases by 10% every year (World Health Organization, 2020b), with a majority of the cases being observed among previously treated patients with TB hampering the global TB control efforts. In 2019, an estimated 3.3% of new TB cases and 17.7% of previously treated cases were diagnosed globally with MDR-TB/rifampicin-resistant TB (RR-TB) (World Health Organization, 2020a). In 2016, an estimated 240,000 death were caused by MDR-TB (World Health Organization, 2020b). In the European Union (EU) and the European Economic Area, the overall TB incidence is 10.2 per 100,000 population with an uneven distribution of the MDR-TB cases throughout the region, ranging from 1%

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to 20% (ECDC, 2020). In Eastern Europe, MDR-TB transmission is prevalent (Acosta et al., 2014), whereas MDR-TB is rare in most Western European countries, with the highest prevalence observed among immigrants (Hargreaves et al., 2017).

Denmark is a low TB incidence country with an annual incidence of 5 cases per 100,000[2]. Generally, the prevalence of drug-resistant (DR)-TB is low with isoniazid (H) monoresistance found in approximately 3% of the cases and approximately 3 MDR-TB cases per year (<1%) (Bang et al., 2010; Statens Serum Institut, 2019). In 2006, the first confirmed transmission of active MDR-TB in Denmark occurred between a foreign-born brother and sister based on identical Mtb genotypes, but, until 2018, MDR-TB transmission was nonexistent except for a few pairwise related cases within families or friends. (Bang et al., 2010)

In this study, we describe and report the first Danish outbreak of MDR-TB that involves 6 Danish-born cases over a period of 10 years, based on genotyping data on all Mtb culture-positive TB cases in the country during the last 3 decades.

Study population and methods

TB detection and treatment

In Denmark, TB diagnostics and treatment are free of charge with universal healthcare paid through taxes. All diagnostics are performed in the International Reference Laboratory of Mycobacteriology (IRLM), at the Statens Serum Institut (SSI), which receives specimens from all patients suspected for TB and other mycobacterioses. Specimens are routinely analyzed by microscopy, PCR, and culturing on solid and liquid media. Culture-positive specimens are genotyped and tested for geno- and phenotypic drug susceptibility. The university hospitals in Copenhagen, Odense, Aarhus, and Aalborg handle the clinical management of MDR-TB cases, centrally coordinated by a national MDR-TB council based on World Health Organization (WHO) recommendations (World Health Organization, 2020c).

In Denmark, genotyping is performed by mycobacterial interspersed repetitive unit-variable number tandem repeat analysis (MIRU-VNTR) (Supply et al., 2006), using Bionumerics software (v7.6; Applied Maths, Sint-Martens-Latem, Belgium). MIRU-VNTR barcodes are translated into MLVA Mtbc15-9 genotype codes using the MIRU-VNTRplus web service (Weniger et al., 2010) assigned to each culture-positive isolate.

For whole-genome sequencing (WGS), the Illumina MiSeq platform is used to generate paired-end (2 × 150 bp) sequencing reads, which are quality trimmed and mapped to the MtbH37Rv reference genome to identify single-nucleotide polymorphisms (SNPs) as previously described (Folkvardsen et al., 2020; Phelan et al., 2019). In addition, the software tools TB-profiler (v2.8.6) generate a genotypic resistance profile, based on known resistance-mutations, and SpoTyping (v2.1) (Xia et al., 2016) performs *in silica* spoligotyping for the sequenced isolates. A phylogenetic overview of the cluster based on aligned SNPs is generated with IQ-TREE software (v1.6.12) (Kalyaanamoorthy et al., 2017; Nguyen et al., 2015) using the built-in automated substitution model selection algorithm and visualized in iTol (v5) (Letunic and Bork, 2019). Mutations conferring resistance to rifampicin and isoniazid are detected by the GenoType MTBDR*plus* assay, followed by FluoroType MTBDR (both Bruker, Nehren, Germany). Phenotypic drug susceptibility is determined by the proportion method on the BD BACTEC™ MGIT™960 system (Beckton Dickinson, New Jersey, United States) (Walters and Hanna, 1996).

TB contact tracing, clinical and epidemiological information

In Denmark, contact tracing is performed for infectious TB cases to identify other cases of active TB and cases of tuberculosis infection (TBI). Early identification of active TB in contacts reduces disease severity and Mtb transmission, and identification of TBI allows for targeted preventive treatment. Contact tracing is supplemented by results from the nationwide genotyping at the SSI. If specific TB clusters are identified, they are addressed on the basis of the principle of concentric circles of exposure (Fox et al., 2013) starting with the closest contacts. The first-line infection control measures include epidemiological interviewing of cases, Quantiferon (Qtf) testing (QuantiFERON-TB Gold PLUS, QIAGEN, Hilden, Germany) and pulmonary x-ray of selected contacts.

Clinical and epidemiological information of the individual cases is reported on a mandatory notification form to the Department of Infectious Disease Epidemiology and Prevention, SSI, which, together with the laboratory, is responsible for TB surveillance in Denmark. In Denmark, TB has been notifiable by law since 1951, with the first mortality records from 1876 (Fight Against Tuberc. Denmark, 1950).

Results

In 2018, 3 MDR-TB cases with resistance to rifampicin (R) and H were identified, initially based on rpoB S450L and katG S315T mutations confirmed phenotypically. Two of these cases were sputum smear-positive and Mtb culture-positive and belonged to the MIRU-VNTR genotype 1220-15. The last case was sputum smear-negative, but Mtb culture-positive, and belonged to the MIRU-VNTR genotype 22299-15, which differs in only 1 locus from 1220-15. Epidemiological contact investigation data showed that all 3 cases lived in the same apartment building and made regular visits to local pubs.

Case 1 (male, aged 47 years) was admitted in February 2018 owing to TB symptoms over several months and a history of poorly controlled diabetes. He completed 12-month 5-drug combination therapy with good adherence and had sputum-negative controls ever since.

Case 2 (male, aged 46 years) was admitted to the department simultaneously with case 1 upon contacting his family physician with symptoms and a history of contact to case 1. He struggled with multiple psychiatric and gastrointestinal disorders and partially completed the initial treatment. He relapsed in February 2020, but by August 2021 he had completed a full 18-month regimen with good adherence. Fortunately, no evolvement of further resistance was seen. A total of 99 people were screened by Qtf-test in relation to cases 1 and 2, resulting in 11 Qtf-positive cases, 4 people completed 6 months of prophylactic latent tuberculosis infection (LTBI) treatment.

Case 3 (male, aged 50 years) visited the emergency department in April 2018 and had a chest x-ray was taken that showed lesions suspicious for TB, but this was not identified, and he was not admitted. On contact tracing, he was identified as a possible MDR-TB case and was admitted to the hospital. He struggled with severe alcohol abuse and had low treatment adherence. The patient only completed 9 months of treatment but has subsequently had several negative sputum controls. Case 3 lived in the same apartment building as cases 1 and 2 but had been out of the country during the contact investigations of the former 2 cases. During follow-up of case 3, 13 people were screened by Qtf-test in relation to the case, and all the results were negative.

By scrutinizing the nationwide genotyping records, a genotypically related TB case from 2010 (case 4, male aged 57 years) (MIRU-VNTR genotype 1220-15) was identified. After returning from extended backpacking under primitive living conditions in India, this person lived under poor conditions in a closed community in Denmark. At the time of admission in 2010, the patient was severely ill and subsequently died from multiple organ failure. Posthumously, the patient was diagnosed with smear-positive MDR-TB with the same genotypic resistance pattern as

observed for the 3 patients later diagnosed in 2018. Subsequent WGS analysis supported the molecular relation between the Mtb strains from the 4 patients. The exact epidemiological link from case 4 in 2010 (the initial case) to the subsequent 3 cases in 2018 remains unknown. However, before 2010, case 1 had regularly bought cannabis from the community where case 4 lived. In 2010, focused x-ray screening of 50 people in the closed community was performed, resulting in 0 extra cases.

Case 5 (male aged 44 years) was admitted to the hospital because of liver cirrhosis in the autumn of 2018 but was diagnosed with MDR-TB after clinical work-up. This happened 6 months after the admission of case 3 to the hospital. Case 5 previously had an inconclusive Qtf-test in 2018 related to the contact investigation of case 1 but had not received preventive treatment. Case 5 and case 1 lived in the same city. The patient was smear-negative but Mtb culture-positive with the same resistance pattern as cases 1-4 and with MIRU-VNTR genotype 1220-15. The patient died from liver cirrhosis before completion of TB treatment. Concerning this case, 105 people were screened by either Qtf-test or Qtf-test + chest x-ray, resulting in 1 Qtf-positive case who completed 6 months of prophylactic LTBI treatment. Simultaneously, 89 people associated with the community where case 4 lived were screened by sputum smear test resulting in 0 cases.

Following the 5 cases, in August 2019, 48 people were screened by a mobile x-ray van specifically targeting frequent visitors to the local pubs and homeless shelters. The screening detected 1 TB-positive, non-MDR case.

The most recent case (case 6, male aged 49 years) from 2019 was smear-positive and sputum Mtb culture-positive with the same resistance pattern as the former 5 cases and MIRU-VNTR genotype 1220-15. In 2018, this patient was identified as a contact to cases 1-3, and 5, as the patient was visiting the same pub but had failed to attend the post-exposure examinations. In 2019, he was admitted to the hospital with bronchiectasis, but found to be TB-positive and subsequently completed TB combination therapy with sputum-negative controls. Through contact tracing, 18 people were screened by Qtf-test, and all results were negative.

Thus, in total, 422 people were screened in the various contact investigations, but apart from the 6 patients who were found Mtb-positive at hospital admissions, none of the screened persons developed MDR-TB with the MDR-TB outbreak strain, although a few of the screened persons were found Mtb-positive with other strains. Among the 6 MDR-TB cases, 5 could be linked epidemiologically to the same apartment building with a pub, whereas the last case had a history of heavy alcohol consumption and was also associated with the pub environment in the same city as cases 1-3. One case (case 4) died before TB diagnosis, 1 case (case 5) died from cirrhosis before completing treatment, 3 cases (case 1, 3, and 6) completed treatment, and 1 case (case 2) has completed retreatment for MDR-TB relapse.

Molecular epidemiology

We used available WGS data from ≈4,500 isolates in the nationwide IRLM strain collection, from 1993 to 2020, to identify possible transmission links to the index case and to explore the immediate genomic neighborhood of the Danish MDR-TB cluster. By including all isolates within 150 SNPs of the index case, we were able to identify 22 additional isolates from 20 patients with TB. These included 10 other known isolates in the strain collection (from 9 patients) with MIRU-VNTR 1220-15 genotype, 7 isolates (from 6 patients) with different but closely related MIRU-VNTR genotypes (19929-15, 7122-15, 22299-15, 1220-28), and 6 isolates without MIRU-VNTR types. The related MIRU-VNTR types only differed from 1220-15 at a single locus. None of the isolates had the same H and R resistance profile as the Danish MDR-TB cluster, but 2 isolates (from a single patient) were H resistant. The average SNP distance between isolates belonging to the Danish MDR-TB cluster was 2.5 SNPs (range: 1-6 SNPs) only, whereas the average distance between members of this cluster to all other isolates was 102 SNPs (range: 74-144 SNPs), ruling out any credible domestic links to case 4. Furthermore, most of the 22 isolates were collected from foreign-born patients, with countries of origin located in South Asia (Afghanistan, Pakistan, Nepal, and India, respectively). We also used the WGS data to construct a phylogenetic tree illustrating the relationships between the 30 isolates (Figure 1). The 30 isolates belong to the East-African-Indian Mtb complex lineage (lineage 3), comprising spoligotypes SIT2145 (the Danish MDR-TB isolates) and SIT26 (all other isolates), which are both members of spoligotype lineage CAS1-Delhi. This particular spoligotype is strongly geographically linked to the particular world region (Couvin et al., 2019), where the index case traveled. Thus, the East-African-Indian origin combined with the short genome distance confirms the relation between the index case and the other cases despite no direct epidemiological link.

Discussion

In this study, we describe the first MDR-TB outbreak in Denmark. Six Danish-born MDR-TB cases were found to be phylogenetically linked in a cluster of East-African-Indian origin. Based on molecular epidemiology, we concluded that the case diagnosed in 2010, hereafter referred to as the primary case, gave rise to the outbreak spanning 2010-2019. The primary case traveled in India before being diagnosed with MDR-TB in 2010, whereas the subsequent cases appeared from 2018 to 2019. The large dispersion in the timespan of this outbreak, from the primary case to the subsequent cases, highlights the benefits of combining routine molecular epidemiological surveillance data from the laboratory with the classic epidemiological case-based surveillance data from notifications and clinical contact investigations data.

It is remarkable, that the primary case appeared 8 years earlier than the subsequent 5 cases, and that this link was discovered through laboratory genotyping surveillance. When the subsequent cases were identified, a routine comparison with previously stored Mtb genotypes identified the likely source of the outbreak. In addition, the genotyping results established the recent connections between the outbreak cases and thus supplemented and directed the contact investigations, all these factors support disease control measures and prevent the further spread of MDR-TB.

Four of the 6 cases in this MDR-TB outbreak were sputum-positive indicating that late diagnosis leads to a higher risk of a severe disease that may potentially result in additional MDR-TB transmission that challenges TB eradication. The time span of 10 years from the first case to the last case in the outbreak highlights the need for increased focus on screening in risk-group environments to enhance early diagnosis and treatment (Kamper-Jørgensen et al., 2012; Lillebaek et al., 2004). In the Danish outbreak, the primary case had no known connection to any of the other patients, indicating transmission within a public place such as the pub. As seen in this study, although uncommon, transmission in modern housing has occasionally been reported (Dawson et al., 2012; Moreau et al., 2012). In general, Mtb transmission by casual contact is uncommon, but cafes and bars are often poorly ventilated and crowded, and transmission of TB in these public places has been described. In a recent study, Klovdahl et al. demonstrated that such locations often represent the common link between patients with recent TB diagnosis where a classic epidemiological link could not be established (Klovdahl et al., 2001). This has also been shown in previous studies where molecular methods could not be corroborated by conventional contact

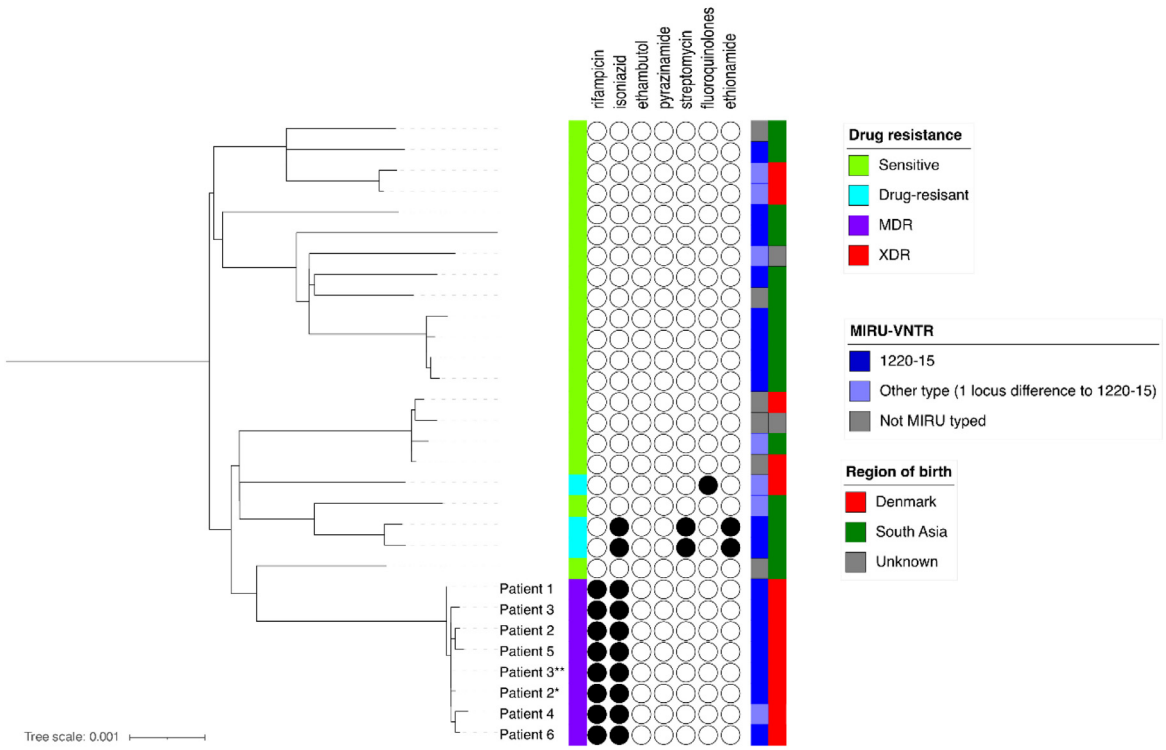

Figure 1. Maximum likelihood tree showing phylogenetic relationships, resistance genotypes, MIRU-subtypes, and regions of origin of IRLM strains clustering within 150 SNPs of the MDR-TB cluster (bottom clade). One patient (*) had 2 sputum samples taken within 2 days of each other, whereas another (**) had recurrent TB after 2 years. Abbreviations: IRLM = International Reference Laboratory of Mycobacteriology; MDR-TB = multidrug-resistant tuberculosis; MIRU = mycobacterial interspersed repeated units; SNP = single-nucleotide polymorphism; TB = tuberculosis.

tracing (Borrell et al., 2009; Daley et al., 2002; Lillebaek et al., 2001; Maguire et al., 2002).

The WHO recommends systematic screening of risk groups in TB low-incidence countries (World Health Organization, 2013). In these countries, TB tends to be concentrated in urban areas, particularly among high-risk groups such as socially marginalized people (de Vries et al., 2014), as also seen in Denmark (Lillebaek et al., 2013; Stærke et al., 2016). However, TB control among marginalized persons, including screening programs, can be very challenging, calling for TB control interventions based on a multisectoral approach targeting the most socioeconomic deprived risk groups in the society using dedicated and trained staff.

Guthrie et al (Guthrie et al., 2018) found that although TB in a low-incidence setting largely arises through reactivation of LTBI in foreign-born persons, local Mtb transmission occurs in discrete populations with distinct disease and risk factor profiles, representing groups for targeted interventions. Active TB screening in high-risk groups in Denmark has shown that it is feasible to screen such groups using spot sputum culture. This has proven a promising alternative to mobile x-ray screening, identifying 80% microscopy negative cases coincident with an early, less infectious stage of the disease, thereby preventing transmission and increasing disease control in high-risk groups (Jensen et al., 2015). In addition, as pointed out by Klovdahl et al (Klovdahl et al., 2001), programs to reduce TB transmission focusing on household members and other close contacts require reconsideration. Modern, multicultural societies encompass different ways of living and different social patterns among groups of people. Therefore, different definitions of close contacts need to be applied for different groups, making contact tracing and TB prevention more efficient. Outbreaks of MDR-TB in countries said to be in the elimination phase of TB, emphasizes the fact that as long as TB remains a major global health problem, no nation can expect to eliminate the disease (Dahle et al., 2003).

In the Danish MDR-TB outbreak, none of the cases had received previous TB treatment. However, we know from previous studies that MDR-TB cases are more likely to be retreatment cases (Faustini et al., 2006; Roberts-Witteveen et al., 2015; Shenoi and Friedland, 2009). As with other bacteria, drug resistance can develop through incomplete, erratic, or inadequate treatment. On the basis of genotyping results, the primary case was infected in India, an MDR-TB high-incidence area, and carried the infection back home to Denmark. Immigration from countries with high rates of MDR-TB is likely to pose an important future challenge for TB control in European countries, although not yet seen often in Denmark (World Health Organization, 2010). In our fellow Nordic countries, MDR-TB remains quite uncommon (ECDC, 2016; Ghebremichael et al., 2008; Vasankari et al., 2012). To our knowledge, Norway is the only Nordic country previously reporting a larger MDR-TB cluster, comprising 20 patients over several years (Dahle et al., 2003). The Norwegian study found the MDR-TB strain to be as transmissible and pathogenic as drug-susceptible strains (Dahle et al., 2003).

Besides the inherent personal risk for patients infected with MDR-TB, this disease also poses a significant strain on health care systems. MDR-TB cases in Europe are reported to have an estimated 9% increased risk of mortality (ECDC, 2020), are difficult to treat and require many resources both in terms of healthcare workers and finances. The Centers for Disease Control and Prevention (CDC) estimates the cost of 1 MDR case to be 8 times that of a pan-susceptible case, whereas the number is 15 for an extensively drug-resistant (XDR) case (Centers for Disease Control and Prevention, 2018). If one imagines that the largest outbreak in Denmark, Cluster 2 (MIRU 1112-15), consisting of more than 1,000 cases and

spanning almost 30 years, had been an MDR outbreak, this outbreak would have meant an estimated expense of 60 million Euros (450 million Danish kroner), based on a review of the costs of TB disease in the EU (Diel et al., 2014). This is approximately 6 times the estimated cost for this outbreak of susceptible TB. This emphasizes the importance of diagnosing and stopping disease outbreaks.

This investigation of a drug-resistant TB cluster illustrates the added value of using routine genotyping to reveal and delimit previously unknown routes of transmission. Hereby, analyzing transmission pathways in the Danish setting, which is high in resources and low in TB incidence, where TB clusters can be dispersed over a significant amount of years with very few cases. Still, genotyping needs to be combined with classical epidemiological methods to establish the true epidemiological links as genotyping cannot stand alone.

Strength and limitations:

The main strength of our study is the mandatory notification of TB cases in Denmark that led to high-quality registers. In the Danish Civil Registration System, all residents in Denmark and persons with residence permits are registered with a unique personal identification number. The use of these numbers allowed us to identify and link individual patients across clinical, epidemiological, and laboratory data, allowing complete follow-up. A study of the completeness of tuberculosis notification systems in 6 EU countries (2014-2016) found the observed and estimated completeness of TB notification in Denmark to be 98.7% and 98.4% respectively, indicating a very low proportion of underreporting (Straetemans et al., 2020). In our study, underreporting would mean that we possibly underestimated the size of this cluster and overlooked other clusters. This is not that likely, because all testing and treatment is free of charge, paid through tax in Denmark, and data on treated patients are linked by the personal identifier as previously mentioned. In addition, patients with MDR-TB would likely be difficult to treat with standard treatment for susceptible TB and thus, ultimately end up with a microbiological diagnosis and be identified through the automatic mandatory laboratory notification system.

Conclusions

This study reports the first Danish outbreak of MDR-TB involving 6 Danish-born cases over a period of 10 years based on clinical-, epidemiological-, and genomic routine data. The long time span between the primary case and subsequent cases illuminates the need for, and advantage of, close collaboration between laboratory-, epidemiological-, and clinical services, potentially revealing unknown transmission routes for the benefit of TB control.

Especially in TB low-incidence countries, MDR-TB outbreaks can be difficult to detect at an early stage, as most clinicians will never encounter the disease. In addition, case finding in this socially vulnerable group at risk of TB demands focused efforts by specially dedicated staff. Contact tracing will occur in relation to the occurrence of a clinical case and normally focus on a limited time span. The addition of molecular genomic typing data can reveal and deliminate previously unknown routes of transmission in low-incidence countries where TB clusters can be dispersed over a significant amount of years.

Declaration of Competing Interest

There are no conflicts of interest to declare pertaining to this work.

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No funding was received for this work.

Ethical approval

The study was reported to the Danish Data Protection Agency through the Compliance Division at Statens Serum Institut. The study was purely register-based and did not involve any study participants. For those reasons and according to Danish legislation, scientific ethical approval was not required.

References

Acosta CD, Dadu A, Ramsay A, Dara M. Public Health Action International Union Against Tuberculosis and Lung Disease Health solutions for the poor Drug-resistant tuberculosis in Eastern Europe: challenges and ways forward. PHA 2014;4:2012–14. doi:10.5588/pha.14.0087.

Bang D, Lillebaek T, Thomsen VO, Andersen ÅB. Scandinavian Journal of Infectious Diseases Multidrug-resistant tuberculosis: Treatment outcome in Denmark 2010;42:288–93. doi:10.3109/00365540903456282.

Borrell S, Español M, Orcau À, Tudó G, March F, Caylà JA, et al. Factors associated with differences between conventional contact tracing and molecular epidemiology in study of tuberculosis transmission and analysis in the city of barcelona, spain. J Clin Microbiol 2009;47:198–204. doi:10.1128/JCM.00507-08.

Centers for Disease Control and Prevention. The Costly Burden of DrugResistant TB Disease in the U.S. CDC FACT SHEET 2018. https://www.cdc.gov/nchhstp/newsroom/docs/factsheets/costly-burden-dr-tb-508.pdf (accessed January 28, 2021).

Couvin D, Reynaud Y, Rastogi N. Two tales: Worldwide distribution of Central Asian (CAS) versus ancestral East-African Indian (EAI) lineages of Mycobacterium tuberculosis underlines a remarkable cleavage for phylogeographical, epidemiological and demographical characteristics. PLoS One 2019;14. doi:10.1371/journal.pone.0219706.

Dahle UR, Sandven P, Heldal E, Mannsaaker T, Caugant DA. Deciphering an Outbreak of Drug-Resistant Mycobacterium tuberculosis. J Clin Microbiol 2003;41:67–72. doi:10.1128/JCM.41.1.67-72.2003.

Daley MF, Steiner JF, Brayden RM, Xu S, Morrison S, Kempe A. Immunization registry-based recall for a new vaccine. Ambul Pediatr 2002;2:438–43. doi:10.1367/1539-4409(2002)002<0438:IRBRFA>2.0.CO;2.

Dawson P, Harris TG, Ahuja SD, Anderson JA, Perri BR. Epidemiology Of Tuberculosis Among New Yorkers Living In Public Housing, 2001-2009. Am j Respir Crit Care Med 2012.

Diel R, Vandeputte J, de Vries G, Stillo J, Wanlin M, Nienhaus A. Costs of tuberculosis disease in the European Union: a systematic analysis and cost calculation. Eur Respir J 2014;43:554–65. doi:10.1183/09031936.00079413.

ECDC Tuberculosis surveillance report 2020; 2020. p. 2016–18.

ECDC. Finland 2016; 2016 https://www.euro.who.int/__data/assets/pdf_file/0011/310043/TB-surveillance-report-2016-Finland.pdf?ua=1.

Faustini A, Hall AJ, Perucci CA. Risk factors for multidrug resistant tuberculosis in Europe: A systematic review. Thorax 2006;61:158–63. doi:10.1136/thx.2005.045963.

Folkvardsen DB, Norman A, Rasmussen EM, Lillebaek T, Jelsbak L, Andersen ÅB. Recurrent tuberculosis in patients infected with the predominant Mycobacterium tuberculosis outbreak strain in Denmark. New insights gained through whole genome sequencing. Infect Genet Evol 2020;80. doi:10.1016/j.meegid.2020.104169.

Fox GJ, Barry SE, Britton WJ, Marks GB. Contact investigation for tuberculosis: A systematic review and meta-analysis. Eur Respir J 2013;41:140–56. doi:10.1183/09031936.00070812.

Ghebremichael S, Petersson R, Koivula T, Pennhag A, Romanus V, Berggren I, et al. Molecular epidemiology of drug-resistant tuberculosis in Sweden. Microbes Infect 2008;10:699–705. doi:10.1016/j.micinf.2008.03.006.

Guthrie JL, Kong C, Roth D, Jorgensen D, Rodrigues M, Hoang L, et al. Molecular Epidemiology of Tuberculosis in British Columbia, Canada: A 10-Year Retrospective Study. Clin Infect Dis 2018;66:849–56. doi:10.1093/cid/cix906.

Hargreaves S, Lönnroth K, Nellums LB, Olaru ID, Nathavitharana RR, Norredam M, et al. Multidrug-resistant tuberculosis and migration to Europe. Clin Microbiol Infect 2017;23:141–6. doi:10.1016/j.cmi.2016.09.009.

Jensen SG, Olsen NW, Seersholm N, Lillebaek T, Wilcke T, Pedersen MK, et al. Screening for TB by sputum culture in high-risk groups in Copenhagen, Denmark: A novel and promising approach. Thorax 2015;70:979–83. doi:10.1136/thoraxjnl-2015-207162.

Kalyaanamoorthy S, Minh BQ, Wong TKF, Von Haeseler A, Jermiin LS. ModelFinder: Fast model selection for accurate phylogenetic estimates. Nat Methods 2017;14:587–9. doi:10.1038/nmeth.4285.

Kamper-Jørgensen Z, Andersen AB, Kok-Jensen A, Kamper-Jørgensen M, Bygbjerg IC, Andersen PH, et al. Migrant tuberculosis: The extent of transmission in a low burden country. BMC Infect Dis 2012;12:60. doi:10.1186/1471-2334-12-60.

Klovdahl AS, Graviss EA, Yaganehdoost A, Ross MW, Wanger A, Adams GJ, et al. Networks and tuberculosis: an undetected community outbreak involving public places. Soc Sci Med 2001;52:681–94. doi:10.1016/S0277-9536(00)00170-2.

Letunic I, Bork P. Interactive Tree of Life (iTOL) v4: Recent updates and new developments. Nucleic Acids Res 2019;47:W256–9. doi:10.1093/nar/gkz239.

Lillebaek T, Andersen AB, Bauer J, Dirksen A, Glismann S, De Haas P, et al. Risk of Mycobacterium tuberculosis transmission in a low-incidence country due

to immigration from high-incidence areas. J Clin Microbiol 2001;39:855–61. doi:10.1128/JCM.39.3.855-861.2001.

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;51:4040–4. doi:10.1128/JCM.01916-13.

Lillebaek T, Dirksen A, Kok-Jensen A, Andersen ÅB. A dominant Mycobacterium tuberculosis strain emerging in Denmark. Int J Tuberc Lung Dis 2004.

Maguire H, Dale JW, McHugh TD, Butcher PD, Gillespie SH, Costetsos A, et al. Molecular epidemiology of tuberculosis in London 1995-7 showing low rate of active transmission. Thorax 2002;57:617–22. doi:10.1136/thorax.57.7.617.

Moreau D, Gratrix J, Kunimoto D, Beckon A, Der E, Hansen E, et al. A shelter-associated tuberculosis outbreak: A novel strain introduced through foreign-born populations. Can J Public Heal 2012;103:408–12. doi:10.1007/bf03405627.

Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ. IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. Mol Biol Evol 2015;32:268–74. doi:10.1093/molbev/msu300.

Phelan JE, O'Sullivan DM, Machado D, Ramos J, Oppong YEA, Campino S, et al. Integrating informatics tools and portable sequencing technology for rapid detection of resistance to anti-tuberculous drugs. Genome Med 2019;11:41. doi:10.1186/s13073-019-0650-x.

Project HOPE. COVID-19 Is On Track To Become 2020's Leading Infectious Disease Killer, Warns Dr. Tom Kenyon 2020. https://www.projecthope.org/covid-19-is-on-track-to-become-2020s-leading-infectious-disease-killer-warns-dr-tom-kenyon/11/2020/(accessed February 16, 2021).

Roberts-Witteveen A, Reinten T, Christensen A, Sintchenko V, Seale P, Lowbridge C. Multidrug-resistant tuberculosis in New South Wales, Australia, 1999-2010: A case series report. Int J Tuberc Lung Dis 2015;19:850–6. doi:10.5588/ijtld.14.0575.

Shenoi S, Friedland G. Extensively drug-resistant tuberculosis: A new face to an old pathogen. Annu Rev Med 2009;60:307–20. doi:10.1146/annurev.med.60.053107.103955.

Stærke NB, Hilberg O, Wejse C, Hoffmann HJ, Fløe A. Latent tuberculosis infection is prevalent among socially marginalised citizens in Aarhus, Denmark. Dan Med J 2016.

. Tuberculosis- 2018 report on disease occurrence, 77. Epi-News; 2019.

Straetemans M, Bakker MI, Alba S, Mergenthaler C, Rood E, Andersen PH, et al. Completeness of tuberculosis (TB) notification: inventory studies and capture-recapture analyses, six European Union countries, 2014 to 2016. Eurosurveillance 2020;25:1. doi:10.2807/1560-7917.ES.2020.25.12.1900568.

Supply P, Allix C, Lesjean S, Cardoso-Oelemann M, Rüsch-Gerdes S, Willery E, et al. Proposal for standardization of optimized mycobacterial interspersed repetitive unit-variable-number tandem repeat typing of Mycobacterium tuberculosis. J Clin Microbiol 2006;44:4498–510. doi:10.1128/JCM.01392-06.

The Fight Against Tuberculosis in Denmark. vol. 22. Nyt Nordisk Forlag Arnold Busck; 1950. 10.1109/MPER.2002.4312181.

Vasankari T, Soini H, Liippo K, Ruutu P. MDR-TB in Finland - still rare despite the situation in our neighbouring countries. Clin Respir J 2012;6:35–9. doi:10.1111/j.1752-699X.2011.00242.x.

de Vries G, Aldridge RW, Caylã JA, Haas WH, Sandgren A, van Hest NA, et al. Epidemiology of tuberculosis in big cities of the European union and European economic area countries. Eurosurveillance 2014;19. doi:10.2807/1560-7917.ES2014.19.9.20726.

Walters SB, Hanna BA. Testing of Susceptibility of Mycobacterium tuberculosis to Isoniazid and Rifampin by Mycobacterium Growth Indicator Tube Method. J Clin Microbiol 1996;34:1565–7.

Weniger T, Krawczyk J, Supply P, Niemann S, Harmsen D. MIRU-VNTRplus: a web tool for polyphasic genotyping of Mycobacterium tuberculosis complex bacteria. Nucleic Acids Res 2010. doi:10.1093/nar/gkq351.

World Health Organization. GLOBAL TUBERCULOSIS REPORT 2020; 2020a http://apps.who.int/bookorders accessed November 23, 2020.

World Health Organization. WHO | Drug-resistant TB: global situation. WHO | Drug-Resistant TB Glob Situat; 2020b https://www.who.int/tb/areas-of-work/drug-resistant-tb/global-situation/en/ accessed November 23, 2020.

WHO consolidated guidelines on tuberculosis. Module 4, Treatment : drug-resistant tuberculosis treatment 2020c. p. 98.

World Health Organization. Systematic screening for active tuberculosis Principles and recommendations; 2013 https://www.who.int/tb/publications/Final_TB_Screening_guidelines.pdf accessed December 9, 2021.

World Health Organization. Multidrug and extensively drug-resistant TB (M/XDR-TB) 2010 GLOBAL REPORT ON SURVEILLANCE AND RESPONSE; 2010 https://apps.who.int/iris/handle/10665/44286 accessed December 14, 2020.

Xia E, Teo YY, Ong RTH. SpoTyping: Fast and accurate in silico Mycobacterium spoligotyping from sequence reads. Genome Med 2016;8:19. doi:10.1186/s13073-016-0270-7.