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Editorial

Tuberculosis in the European Union/European Economic Area: much progress, still many challenges

Jean-Paul Guthmann¹, Walter Haas²
1. Santé publique France, Saint-Maurice, France
2. Robert Koch Institute, Berlin, Germany
Correspondence: Jean-Paul Guthmann (Jean-Paul.GUTHMANN@santepubliquefrance.fr)


Tuberculosis (TB) is still a deadly disease in 2019. It ranks among the top 10 causes of death globally and is the most frequent cause of death from a single infectious agent. TB remains a major public health problem worldwide, with the highest disease burden in low- and middle-income countries; according to the World Health Organization (WHO), an estimated 10 million people fell ill with tuberculosis in 2017, 1.5 million of whom died [1]. Children under 5 years of age are especially at risk of developing severe disease manifestations such as meningitis or disseminated disease and are therefore at higher risk of death and sequelae. In addition, young children with untreated latent TB infection (LTBI) or disease may experience reactivated TB later in life, which poses a major obstacle for elimination of the disease.

A total of 3% of global TB cases occur in the WHO European Region [1]. From a global perspective, the European Union/European Economic Area (EU/EEA)—which comprises 31 of the 53 countries in the Region—is making much progress, with decreasing numbers of people getting sick from TB or dying from the disease [2]. Despite this improvement, several problems remain that need to be considered. In the EU/EEA, the frequency and burden of TB varies considerably between countries, with country-specific notification rates ranging from 2.6 in Liechtenstein to 66.2 per 100,000 in Romania [2]. Within the EU/EEA, TB is largely a disease of the poor, affecting the most vulnerable and impoverished population groups. In Portugal, for example, unemployed individuals were shown to be at higher risk of developing TB [3]. In France, the TB notification rate is 10 times higher in foreign-born individuals compared with the native population, and in homeless individuals this rate was 166 per 100,000 in 2015 [4]. Though the EU/EEA has a relatively low incidence of TB compared to the rest of the world, thousands of people still die from the disease every year, even though it can usually be cured by timely treatment with an adequate and complete drug regimen. The high number of multidrug-resistant (MDR) TB cases in some countries remains a constant threat for local populations and, because of travel and migration, also puts at risk those living in bordering countries and other parts of Europe. At 3.8%, the proportion of MDR TB in Germany in 2017 was almost four times higher in foreign-born individuals compared to those born in Germany (1.0%); for those born in the newly independent states of the former Soviet Union, this proportion was almost 20 times higher (19.3%) [5].

The present issue of Eurosurveillance features two articles that discuss issues that the EU/EEA is facing regarding TB control. The incidence of TB in most EU/EEA countries is decreasing and this is encouraging. However, one important question is whether the current rate of decrease is enough to meet the targets that all WHO Member States committed to in 2015 to reduce cases of TB and deaths from the disease [6]. This point is discussed by Merk et al., who describe the trends of TB incidence and deaths reported in the EU/EEA during the last decade [7]. The authors show that despite a clear annual decline in cases and deaths, the trend is not enough to reach the set objectives. They also point out that some countries are progressing towards ending TB faster than others, and underline the need to adapt prevention and control measures to a particular country’s situation. Despite substantial improvements in TB control in the EU/EEA, and the fact that the disease is progressively becoming rare in many countries, Merk et al. highlight that further progress in controlling and finally eliminating this severe disease will require a constant effort that is continually adapted to suit distinct geographical areas and the most affected population groups.

Treatment outcome of TB patients and factors that may influence treatment success are further key points in TB control, and these are discussed in an article by Karo...
et al. in this issue of *Eurosurveillance* [8]. Adequate treatment of a TB case cures the patient, rapidly limits the risk of transmission of *Mycobacterium tuberculosis* to close contacts in the family and community, and prevents the development of resistance to anti-TB drugs. Therefore, assessing patients’ treatment outcome remains essential for evaluating national TB control programs; further, identifying factors associated with an unfavourable outcome may help to target control measures in groups that are most at need. In their analysis of factors influencing treatment outcome of TB in Europe, Karo et al. showed an almost nine times higher risk of unsuccessful treatment for patients with MDR TB (Odds ratio (OR): 8.7; 95% confidence interval (CI): 5.09–14.97) [9]. The retrospective analysis in this issue used information on treatment outcome compiled by the European Centre for Disease Prevention and Control (ECDC) in the European Surveillance System (TESSy) database to investigate the association between isoniazid (INH) mono-resistance and TB treatment success. The results show that treatment success did not meet the objectives set by WHO in 2014 [6] and that treatment success for INH mono-resistant TB was significantly lower compared with fully drug-susceptible TB. The authors compare their results to those already published and conclude that increased efforts should be made towards timely detection and management of INH mono-resistant TB, which is frequently underestimated. Timely drug sensitivity testing of all cases and provision of treatment regimens adapted to the strain profile are essential components of TB control that contribute to maintaining a low prevalence of INH mono-resistance observed in the EU/EEA [10]. The timely identification and management of patients infected with a strain resistant to INH, one of the most important first-line drugs for the treatment of TB, should also prevent further development of MDR TB, which remains low overall in most countries of the EU/EEA [2].

It is clear that ending the TB epidemic poses tremendous challenges and requires a concerted international effort. Following the 1993 WHO alert declaring that TB was a global emergency [11], several initiatives (e.g. Stop TB Partnership), sources of funding (e.g. The Global Fund) and political declarations have shown that the international community is committed to the global goal of ending the TB epidemic. In November 2017, the first WHO global ministerial conference on ending TB was held in the Russian Federation and brought together 75 ministers of health, resulting in the Moscow Declaration to End TB [12]. The conference recognised that today TB is the most deadly infectious disease in the world, with considerable economic and social consequences. On 26 September 2018, the United Nations first high-level meeting on TB in New York highlighted the need for immediate action to accelerate progress towards the goal of ending the TB epidemic by 2030. In the political declaration, national leaders committed to taking specific actions against TB [13].

The elimination of TB in the EU/EEA, where a growing number of countries are progressively entering the low-incidence category, poses several challenges, as illustrated by the two articles presented in this issue of Eurosurveillance. The elimination of TB in this region will require additional efforts and specific actions that have been adapted to local epidemiology [14]. Treatment outcome needs to improve, particularly in drug-resistant TB cases, including cases with MDR TB, which is associated with the highest rates of unsuccessful treatment [9]; in order to achieve this, a wider use of rapid molecular testing and the adaptation of treatment regimens are necessary. Continual involvement of TB professionals and close collaboration between clinicians, microbiologists and epidemiologists working in the field are also needed. Immediate contact investigations performed around each newly detected case will help to prevent the occurrence of new cases. The use of genotyping methods to identify related cases and to understand chains of transmission should further help to get closer to a zero transmission goal.

In low-incidence countries, the majority of TB cases are generated through reactivation of latent TB infections (LTBI) acquired abroad [14]. As global migration has increased considerably in recent decades [15,16] and a significant proportion of TB cases in most EU/EEA countries are born in countries with a high incidence of TB [2], early detection and access to health services and care in this specific group should be placed as one of the top priorities of TB control. In addition, individuals with LTBI represent an important reservoir, as they may later progress to TB disease, therefore contributing to future TB burden. In the WHO European Region the prevalence of LTBI has been estimated at 13.7%; this prevalence is 0.3% for recent infections [17], which have the highest risk of progressing towards TB. An important challenge for European control programs is therefore to establish a programmatic approach to LTBI management that takes into account the TB epidemiology in various vulnerable groups, as well as the health system structure, resource allocation and political commitment [18]. This underlines that there is not one single issue to address, but rather that a strategic, comprehensive approach needs to be developed in order to meet the challenge of TB elimination.

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**Conflict of interest**

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References


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Will we reach the Sustainable Development Goals target for tuberculosis in the European Union/European Economic Area by 2030?

Hanna Merk¹, Csaba Ködmön¹, Marieke J van der Werf¹
1. European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden
Correspondence: Hanna Merk (hanna.merk@ecdc.europa.eu)

We assessed progress towards the Sustainable Development Goals target for tuberculosis in the European Union/European Economic Area using the latest tuberculosis (TB) surveillance and Eurostat data. Both the TB notification rate and the number of TB deaths were decreasing before 2015 and the TB notification rate further declined between 2015 and 2017. With the current average decline in notification rate and number of TB deaths however, the EU/EEA will not reach the targets by 2030.

In 2015, all United Nations Member States adopted the 17 Sustainable Development Goals (SDGs) and their 169 targets [1]. The target for tuberculosis (TB) is to end the epidemic by 2030. The End TB Strategy provides three additional sub-targets that are used to measure progress towards the SDGs [2]. According to these sub-targets, the TB incidence should be 80% lower in 2030 compared with 2015; the number of TB deaths should be 90% lower and no family should face catastrophic costs due to TB. Here, we assess progress towards the first two sub-targets at European Union/European Economic Area (EU/EEA) level. Information on catastrophic costs is not available at EU/EEA level.

Analysis
We used data obtained from the European tuberculosis surveillance network under the joint coordination of the European Centre for Disease Prevention and Control (ECDC) and World Health Organization (WHO) Regional Office for Europe [3] and data from Eurostat [4] for the years 2008–17. The TB case data were extracted from The European surveillance system (TESSy) [5] hosted by ECDC (as at 5 October 2018). Since Croatia did not report case-based TB data to TESSy for 2008–11, Croatia was excluded from the notification rate for those years. The population denominators for the notification rates were obtained from Eurostat (as at 5 October 2018), as were the data on cause of death due to TB (ICD 10 code A15-A19 and B90, as at 21 November 2018) [6]. Cause of death data were only available up to 2015 (last updated by EUROSTAT on 20 July 2018). The TB notification rates were used as proxy for TB incidence and reported TB deaths as a proxy for actual number of deaths due to TB.

Countries with missing annual data on deaths and reporting 10 or less deaths per year in the remaining years were considered to have zero TB deaths for the years with missing data. Denmark did not report any data on TB deaths in 2010 but reported more than 10 deaths in the other years. We therefore estimated the number of TB deaths, by calculating the average of the two preceding and following years and applying the ceiling function in STATA version 14.2 (StataCorp, College Station, Texas, United States).

To assess whether the EU/EEA will reach the SDG target we used the average annual change in notification rate between 2008 and 2017 and the average annual change in number of deaths between 2008 and 2015 and assumed that the change will continue similarly in future.

For our analysis, we used STATA/SE 14.2.

Key findings
The total TB notification rate declined during the study period (Figure 1). In 2017, the notification rate was 10.7 per 100,000 population in the EU/EEA, resulting in an overall decline of 10% since 2015. The average annual decline between 2008 and 2017 was 4.8%.

An 80% reduction in TB notifications in the EU/EEA in 2030 compared with 2015 results in a target TB notification rate of 2.4 per 100,000 population.

If the 4.8% average annual decline continued unchanged, the EU/EEA would reach a TB notification...
rate of 5.7 per 100,000 population in 2030. The annual average decline required to reach the target is 10.9%.

The total number of TB deaths declined during the study period (Figure 2). In 2015, the number of registered TB deaths was 4,437. Progress since 2015 cannot be measured as there is no available data after 2015 at EU/EEA level. The average annual decline between 2008 and 2015 was 5.3%.

A 90% reduction in TB deaths in the EU/EEA in 2030 compared with 2015 results in a target of 444 TB deaths per year.

If the 5.3% average annual decline continued unchanged, the EU/EEA would reach 1,947 TB deaths per year in 2030. The annual average decline required to reach the target is 14.2%.

Discussion

The targets for TB incidence and the number of TB deaths set in the End TB Strategy translate to 2.4 TB cases per 100,000 population and 444 TB deaths for the EU/EEA in 2030. Both the annual TB notification rate and the number of TB deaths were decreasing before 2015 and the TB notification rate further declined by 16% between 2015 and 2017. If the average 4.8% annual decline of the TB notification rate continues in the EU/EEA we will not reach the target by 2030; the average 5.3% decline of TB deaths is also not sufficient to reach the target.

Compared to other parts of the world, the observed TB notification rate and number of TB deaths in the EU/EEA are low [7]. Nonetheless, the SDG and End TB Strategy targets apply to the EU/EEA and our results show that there is little room for complacency.

Globally, the average annual decline of the TB incidence rate was 1.5% between 2000 and 2017, far less than what was observed in the EU/EEA [7]. The global number of TB deaths among HIV-negative people decreased by 5% since 2015 and by 29% between 2000 and 2017 [7]. Since 33% of the reported TB cases in the EU/EEA are diagnosed in individuals of foreign origin [8], a decrease in the global incidence of TB may impact the observed TB incidence in the EU/EEA. This would be more apparent in countries that diagnose a large proportion of their TB cases in individuals of foreign origin such as Malta, Norway and Sweden (85% of TB cases of foreign origin).

Within the EU/EEA, some countries are closer to ending TB than others: 24 countries reported a notification rate of less than 10 TB cases per 100,000 population [8]. In addition, there are substantial differences in the annual change in the TB notification rate in the EU/EEA [8]. In the period 2013–17, two countries observed an increasing notification rate of more than 5% per year, 17 had a decreasing notification rate of more than 5% and in 11, the annual change was between -5% and +5%. To reach the target of 2.4 TB cases per 100,000 population, different strategies may need to be applied within the EU/EEA countries, depending on the respective epidemiological situation. Similarly, the estimated TB deaths among HIV-negative persons in EU/EEA countries in 2017 ranged between zero and 920, with an annual percentage change ranging from -17.6% to 15.0% between 2013 and 2017 [8]. Thus further indicating that some EU/EEA countries are making more progress towards the target than others are.

The End TB Strategy includes a package of interventions that are encouraged for use by countries to prevent and control TB and reach the targets [2]. The interventions are grouped under three pillars: (i) integrated, patient-centred care and prevention, (ii) bold policies and supportive systems, and (iii) intensified
It is acknowledged that specific actions are needed in countries that are close to ending TB and aiming for TB elimination [10]. These countries will often have epidemics concentrated in hard-to-reach and vulnerable populations e.g. migrants, prisoners and homeless people. Targeting hard-to-reach and vulnerable populations requires specific interventions and may need additional resources [11-14]. For example, screening and providing treatment for latent TB infection (LTBI) prevents new TB cases [15,16], as well as screening and treating prisoners and migrants for active TB may also contribute to a further decline [12,13]. To our knowledge, however, not all EU/EEA countries test hard-to-reach populations for LTBI. Mathematical modelling and cost-effectiveness studies show that programmatic management of LTBI can have an impact on TB burden [17,18]. In addition, migrants are not screened for TB in all EU/EEA countries [19]. The interventions suggested in the ECDC guidance on TB control in vulnerable and hard to reach populations are also not implemented in all countries in the EU/EEA [11].

Our results come with limitations. We used notification rate as a proxy for TB incidence. We believe this to be a valid approach since several studies have shown that completeness of TB surveillance data in EU/EEA countries is >80% [20-22]. We relied on the completeness and accuracy of the cause of death register for the number of TB deaths. The quality of death registration systems has been assessed as good in most countries of the WHO European Region [23]. We therefore consider our results valid for assessing progress towards the targets on an EU/EEA level. However, accurately assigning cause of death is challenging [24] and improvements in cause of death registration may still be needed on country level [25]. We recognise that improvements in both TB surveillance and cause of death registration can affect the progress assessment if more complete data become available.

In conclusion, additional interventions need to be implemented to reach the targets for TB incidence and number of TB deaths in the EU/EEA, and thus the SDG, especially in countries that are currently facing stable or increasing trends.

Conflict of interest
None declared.

Authors’ contributions
CK, HM and MvdW all contributed to the study plan, writing, reviewing and revising of the manuscript and approved the final draft. CK and HM conducted the analysis.

References


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In January 2019, two influenza A(H3N2) viruses carrying an I38T substitution in the polymerase acidic subunit (PA), which confers reduced susceptibility to baloxavir, were detected from epidemiologically unrelated hospitalised children in Japan. The viruses exhibited reduced susceptibility to baloxavir but were susceptible to neuraminidase inhibitors. Only one of the two children had been treated with baloxavir. An epidemiological analysis suggests possible transmission of the PA I38T mutant A(H3N2) virus among humans.

The cap-dependent endonuclease inhibitor baloxavir marboxil became available in Japan in March 2018 for the treatment of influenza virus infection in patients aged 12 years and older and children younger than 12 years weighing at least 10 kg. Between October 2018 and January 2019, baloxavir was supplied to medical institutions that together serve ca 5.5 million people. In December 2018, we detected influenza A(H3N2) viruses exhibiting reduced susceptibility to baloxavir due to a polymerase acidic subunit I38T substitution detected from a hospitalised child without prior baloxavir treatment, Japan, January 2019.

Detection of polymerase acidic subunit I38T mutant influenza A(H3N2) viruses from hospitalised children

In January 2019, we isolated two influenza A(H3N2) viruses, A/YOKOHAMA/87/2019 and A/YOKOHAMA/88/2019, from two hospitalised children (Table 1). Prior to hospitalisation and virus isolation, both children had received antiviral treatment against influenza. The primary-school child aged 6 years who was infected with A/YOKOHAMA/87/2019 had been treated with a single oral dose of baloxavir on the day of symptom onset and fever resolved within one day of baloxavir administration. Face oedema had developed 2 days after baloxavir administration, although this patient had no underlying diseases. The child was diagnosed with nephritis and hospitalised. The pre-school child aged 5 years who was infected with A/YOKOHAMA/88/2019 had received oseltamivir 3 days after onset of illness, although its clinical benefit is greatest when administered within 48 hours of illness onset. Fever tended to resolve after oseltamivir administration. This child had no underlying diseases but was subsequently hospitalised for pneumothorax and subcutaneous emphysema. No epidemiological link was identified between these patients.

Deep sequencing analysis of the isolates using MiSeq (Illumina, San Diego, California, United States) suggested a possible transmission of the PA I38T mutant A(H3N2) virus among humans.
States) revealed that A/YOKOHAMA/87/2019 and A/YOKOHAMA/88/2019 possessed the PA I38T substitution. These PA I38T mutant viruses possessed different PA sequences and therefore originated from different sources of infection. PA I38 is highly conserved in influenza A and B viruses [1,2]. The I38T substitution was not detected in the Influenza Research Database (www.fludb.org) including 17,227 PA sequences from A(H3N2) viruses until December 2018 [1] or during surveillance studies of baloxavir susceptibility of influenza viruses in Japan (2017/18 influenza season) and the United States prior to the introduction of baloxavir (2016/17 and 2017/18 seasons) [3,4]. Therefore, previous studies concluded that the PA I38T substitution was a baloxavir treatment-emergent substitution [1,2]. The patient infected with A/YOKOHAMA/87/2019 had been treated with baloxavir, indicating the possible emergence of the PA I38T mutant virus under the selective pressure of this drug. In contrast, the patient infected with A/YOKOHAMA/88/2019 was treated with oseltamivir. Usage of baloxavir increased in this influenza season in Japan and an influenza outbreak occurred in the preschool attended by the 5 year-old before this patient's symptom onset, suggesting a possible acquisition of the PA I38T mutant virus by human-to-human transmission.

### Table 1

Influenza A(H3N2) viruses detected from hospitalised children, Japan, January 2019 (n = 2)

<table>
<thead>
<tr>
<th>GISAID isolate ID</th>
<th>Isolate name</th>
<th>Age in years</th>
<th>Onset of symptoms</th>
<th>Antiviral treatment</th>
<th>Day of hospitalisation</th>
<th>Specimen collection</th>
<th>PA substitutiona</th>
<th>Clinical specimen</th>
<th>Virus isolate</th>
</tr>
</thead>
</table>

GISAID: Global Initiative on Sharing All Influenza Data; ID: identity; PA: polymerase acidic subunit.

a For deep sequencing analysis, the mean sequencing depth, threshold used and limit of quantitation used were 14,200, 5% and 2, respectively.

### Table 2

Susceptibility of influenza A(H3N2) viruses detected from hospitalised children, Japan, January 2019 (n = 2)

<table>
<thead>
<tr>
<th>Isolate name</th>
<th>PA substitution</th>
<th>IC50, nM</th>
<th>Baloxavir</th>
<th>Neuraminidase inhibitors (WHO criteria b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Baloxavir</td>
<td>Oseltamivir</td>
</tr>
<tr>
<td>A/YOKOHAMA/87/2019</td>
<td>I38T</td>
<td>157.87</td>
<td>0.52 (NI)</td>
<td>0.16 (NI)</td>
</tr>
<tr>
<td>A/YOKOHAMA/88/2019</td>
<td>I38T</td>
<td>218.89</td>
<td>0.44 (NI)</td>
<td>0.13 (NI)</td>
</tr>
</tbody>
</table>

IC50: 50% inhibitory concentration; PA: polymerase acidic subunit; WHO: World Health Organization.

a The median IC50 values of A(H3N2) viruses isolated in the 2018/19 influenza season in Japan to baloxavir (n = 22 viruses without the PA I38T substitution) and to oseltamivir, peramivir, zanamivir and laninamivir (n = 69) were 3.22 ± 2.93, 0.22 ± 0.15, 0.10 ± 0.03, 0.48 ± 0.27 and 0.92 ± 0.24, respectively.

b NI: normal inhibition.

Antiviral susceptibilities of the polymerase acidic subunit protein I38T mutant viruses

We determined the susceptibilities of the PA I38T mutant viruses to baloxavir and four neuraminidase (NA) inhibitors approved in Japan: oseltamivir, laninamivir, peramivir and zanamivir (Table 2). Antiviral susceptibilities were determined by using a focus reduction assay and a fluorescent NA inhibition assay with the NA-Fluor Influenza Neuraminidase Assay Kit (Applied Biosystems, Carlsbad, California, United States) as previously described [3]. The hydrolysed active form of baloxavir marboxil (baloxavir acid) was purchased from MedChemexpress (Monmouth Junction, New Jersey, United States). Oseltamivir carboxylate, peramivir, and zanamivir were purchased from Sequoia Research Products (Pangbourne, Reading, United Kingdom), and laninamivir was provided by Daiichi Sankyo Co., Ltd. (Tokyo, Japan). Results are expressed as the 50% inhibitory concentration (IC50) values, which were calculated by using MikroWin 2000 software (Mikrotek Laborsysteme GmbH, Overath, Germany). To interpret the NA inhibitor susceptibility, the World Health Organization (WHO) criteria based on the fold change of IC50 values compared with reference IC50 values were applied [5]. These define inhibition of influenza A viruses as normal (<10-fold increase), reduced (10–100-fold increase) or highly reduced (>100-fold increase).
The IC₅₀ values of the viruses to baloxavir and the NA inhibitors are shown in Table 2. Both PA I38T mutant viruses showed normal inhibition with all four NA inhibitors, but exhibited 49- and 68-fold higher IC₅₀ values to baloxavir compared with the median IC₅₀ value of A(H3N2) viruses isolated in the 2018/19 influenza season in Japan. No amino acid substitutions associated with reduced susceptibility to NA inhibitors were detected in either virus. These results indicate that the PA I38T mutant viruses had reduced susceptibility to baloxavir, but remained susceptible to NA inhibitors.

### Discussion

In this study, we detected two PA I38T mutant A(H3N2) viruses respectively from two hospitalised children. In addition, during our nationwide monitoring, we detected nine PA I38T or I38M mutant A(H3N2) viruses from baloxavir-treated patients (Table 3). All of these viruses were isolated in humanised MDCK cells, hCK cells, which express high levels of α₂, 6-sialoglycans and very low levels of α₂, 3-sialoglycans [6]. Deep sequencing analysis revealed that eight of these viruses possessed mixed PA I38T/I or I38T/M/I substitutions in the clinical specimens and six of these eight possessed increased proportion of the PA I38T or I38M substitution after virus isolation. A previous study reported that influenza A/Victoria/3/75(H3N2) viruses with the PA I38T, I38M, or I38F substitutions showed less growth capability than the wild-type virus in cell culture [2]. In contrast, our results indicate that recently circulating A(H3N2) viruses with the PA I38T or I38M substitution grow well, at least in cell culture.

Of the two children described as infected with a PA I38T mutant virus in this report, one (infected with A/YOKOHAMA/88/2019) had not received baloxavir treatment. Because an influenza outbreak, with possible use of baloxavir, had occurred in this child’s preschool prior to their symptom onset, it could be that the child acquired the mutant virus there. This might indicate that the recently circulating A(H3N2) viruses with the PA I38T substitution have, to some extent, retained replication and possible transmission capability in humans. In this respect, the parents, and a sibling were also diagnosed with influenza 4, 5, or 6 days, respectively, after this child’s symptom onset. Moreover, concerning the second child affected by a mutant virus in this study (i.e. the patient infected with A/YOKOHAMA/87/2019), a sibling of this child was diagnosed with influenza 2 days after the child’s

### Table 3

<table>
<thead>
<tr>
<th>GISAID isolate ID</th>
<th>Isolate name</th>
<th>Age in years</th>
<th>Antiviral treatment</th>
<th>Specimen collection</th>
<th>PA substitution</th>
<th>Virus isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPI_ISL_332908</td>
<td>A/YOKOHAMA/133/2018</td>
<td>6</td>
<td>3 Dec 2018</td>
<td>Baloxavir</td>
<td>6 Dec 2018</td>
<td>I38T</td>
</tr>
<tr>
<td>EPI_ISL_332910</td>
<td>A/YOKOHAMA/135/2018</td>
<td>7</td>
<td>4 Dec 2018</td>
<td>Baloxavir</td>
<td>7 Dec 2018</td>
<td>I38T/I mix (T: 80%)</td>
</tr>
<tr>
<td>EPI_ISL_340687</td>
<td>A/KANAGAWA/Ic1807/2018</td>
<td>14</td>
<td>17 Dec 2018</td>
<td>Baloxavir</td>
<td>20 Dec 2018</td>
<td>I38T/I mix (T: 10%)</td>
</tr>
<tr>
<td>EPI_ISL_337453</td>
<td>A/KANAGAWA/AC1817/2018</td>
<td>8</td>
<td>21 Dec 2018</td>
<td>Baloxavir</td>
<td>25 Dec 2018</td>
<td>I38T/M/I mix (M: 8%, M: 20%)</td>
</tr>
<tr>
<td>EPI_ISL_340692</td>
<td>A/KANAGAWA/Ic1817/2019</td>
<td>9</td>
<td>5 Jan 2019</td>
<td>Baloxavir</td>
<td>8 Jan 2019</td>
<td>I38M/M/I mix (M: 8%)</td>
</tr>
<tr>
<td>EPI_ISL_337460</td>
<td>A/KANAGAWA/Ic1827/2019</td>
<td>5</td>
<td>9 Jan 2019</td>
<td>Baloxavir</td>
<td>12 Jan 2019</td>
<td>I38T/I mix (T: 16%)</td>
</tr>
<tr>
<td>EPI_ISL_340690</td>
<td>A/KANAGAWA/AC1829/2019</td>
<td>4</td>
<td>12 Jan 2019</td>
<td>Baloxavir</td>
<td>15 Jan 2019</td>
<td>I38T/I mix (T: 18%)</td>
</tr>
<tr>
<td>EPI_ISL_340695</td>
<td>A/YOKOHAMA/56/2019</td>
<td>1</td>
<td>11 Jan 2019</td>
<td>Baloxavir</td>
<td>15 Jan 2019</td>
<td>I38T</td>
</tr>
<tr>
<td>EPI_ISL_340699</td>
<td>A/YOKOHAMA/61/2019</td>
<td>4</td>
<td>21 Jan 2019</td>
<td>Baloxavir</td>
<td>25 Jan 2019</td>
<td>I38T/I mix (T: 65%)</td>
</tr>
</tbody>
</table>

GISAID: Global Initiative on Sharing All Influenza Data; ID: identity; PA: polymerase acidic subunit.

For deep sequencing analysis, the mean sequencing depth, threshold used and limit of quantitation used were 14,200, 5% and 2, respectively.
symptom onset. Although we could not obtain specimens from family members of either children, these observations could point to a possible transmission of the PA I38T mutant A(H3N2) viruses among humans.

Among the 11 persons infected with PA I38T or I38M mutant A(H3N2) viruses in the 2018/19 season in Japan, all but one were children younger than 12 years. In Phase III clinical trials of baloxavir marboxil, the PA I38T and I38M substitutions emerged in 36 (9.7%) of 370 A(H3N2) viruses obtained from patients aged 12–64 years and in 18 (23.4%) of 77 A(H3N2) viruses obtained from children aged 6 months to 12 years [2,7]. Our results confirm that the incidence of the PA I38 mutant viruses in children younger than 12 years is higher than that in patients aged 12–64 years. Baloxavir was approved in the United States in October 2018 for the treatment of acute uncomplicated influenza A and B infections in patients 12 years and older. Since treatment of children younger than 12 years with baloxavir is approved only in Japan at this time, we believe it is important to share our findings.

Koszalka et al. reported that influenza viruses circulating in the Asia-Pacific region between 2012 and 2018 were susceptible to baloxavir [8]. In the United States, the frequency of reduced susceptibility to baloxavir (> threefold change) due to a PA I38M substitution was 0.032% for A(H3N2) viruses during the 2016/17 and 2017/18 seasons [4]. In Japan, the frequency of A(H3N2) viruses with any PA I38 substitutions identified as playing a role in baloxavir resistance was 0% in the 2017/18 season; however, it increased between September 2018 and February 2019, with all patients, except one, being treated with baloxavir before specimen collection [9]. Therefore, the baloxavir susceptibility of influenza viruses should be closely monitored.

Acknowledgements

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Conflict of interest

None declared.

Authors’ contributions

Designed the analyses: ET, SW, TO. Analysed and interpreted the data: ET, CK, RO, HMo, SF, MS, HMi, KN, NK, TK, AO, HT, AS, KM, TA, MI, MY, SW, TO. Drafted the article: ET. Revised the article: SW, TO.

References


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Research

Isoniazid (INH) mono-resistance and tuberculosis (TB) treatment success: analysis of European surveillance data, 2002 to 2014

Basel Karo1,2,3,4, Anke Kohlenberg4,5, Vahur Hollo⁵, Raquel Duarte⁶, Lena Fiebig⁷, Sarah Jackson⁸, Cathriona Kearns⁹, Csaba Ködmön⁵, Maria Korzeniewska-Kosela 10, Dimitrios Papaventsis11, Ivan Solovic12, Dick van Soolingen13, Marieke J. van der Werf5

1. EPIET: European Programme of Intervention Epidemiology Training, European Centre for Disease Prevention and Control, Stockholm, Sweden
3. Infectious Disease Department, Robert Koch Institute, Berlin, Germany
4. These authors contributed equally to this article and share first authorship
5. European Centre for Disease Prevention and Control, Stockholm, Sweden
6. Directorate General of Health, Lisbon, Portugal
7. Apopo, Sokoine University of Agriculture, Morogoro, Tanzania
8. Health Protection Surveillance Centre, Dublin, Ireland
9. Public Health Agency, Belfast, Northern Ireland
10. National Tuberculosis and Lung Diseases Research Institute, Warsaw, Poland
11. National Reference Laboratory for Mycobacteria, ‘Sotiria’ Chest Diseases Hospital, Athens, Greece
13. Tuberculosis Reference Laboratory, National Institute for Public Health and the Environment, Bilthoven, The Netherlands

Correspondence: Anke Kohlenberg (anke.kohlenberg@ecdc.europa.eu)

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Introduction: Isoniazid (INH) is an essential drug for tuberculosis (TB) treatment. Resistance to INH may increase the likelihood of negative treatment outcome. Aim: We aimed to determine the impact of INH mono-resistance on TB treatment outcome in the European Union/European Economic Area and to identify risk factors for unsuccessful outcome in cases with INH mono-resistant TB. Methods: In this observational study, we retrospectively analysed TB cases that were diagnosed in 2002–14 and included in the European Surveillance System (TESSy). Multilevel logistic regression models were applied to identify risk factors and correct for clustering of cases within countries. Results: A total of 187,370 susceptible and 7,578 INH mono-resistant TB cases from 24 countries were included in the outcome analysis. Treatment was successful in 74.0% of INH mono-resistant and 77.4% of susceptible TB cases. In the final model, treatment success was lower among INH mono-resistant cases (Odds ratio (OR): 0.7; 95% confidence interval (CI): 0.6–0.9; adjusted absolute difference in treatment success: 5.3%). Among INH mono-resistant TB cases, unsuccessful treatment outcome was associated with age above median (OR: 1.3; 95% CI: 1.2–1.5), male sex (OR: 1.3; 95% CI: 1.1–1.4), positive smear microscopy (OR: 1.3; 95% CI: 1.1–1.4), positive HIV status (OR: 3.3; 95% CI: 1.6–6.5) and a prior TB history (OR: 1.8; 95% CI: 1.5–2.2). Conclusions: This study provides evidence for an association between INH mono-resistance and a lower likelihood of TB treatment success. Increased attention should be paid to timely detection and management of INH mono-resistant TB.

Introduction

Tuberculosis (TB) causes a large degree of suffering and an estimated 1.3 million deaths per year globally, occurring mainly in less affluent countries, but also in upper-middle and high-income countries in the European Union/European Economic Area (EU/EEA) [1,2]. In Europe, there has been a steady decline in TB notification rates of ca 5% per year. Nevertheless, TB remains a considerable problem because of multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB [3].

The main public health response to the TB epidemic consists of early diagnosis, prevention of transmission and adequate treatment. In general, treatment is most successful when there is no resistance to any of the drugs designated for treatment of TB [4], and the drugs isoniazid (INH) and rifampicin (RIF) can be included in the treatment regimen. INH has long been an essential component of first-line treatment for active TB and an important drug in TB control because of its potent early bactericidal activity, low rate of adverse events and low cost [5]. Currently, there is no equivalent alternative
INH mono-resistance increases the likelihood of negative treatment outcome and progression to MDR TB [4,8,9]. Reported treatment outcome seems to differ by setting and region for cases with INH-resistant TB [10-12]. Population groups that are especially at risk of negative treatment outcome due to INH mono-resistance are children and HIV-positive patients [13-15]. Recently, two systematic reviews assessed treatment options for INH mono-resistant TB [8,16]. One review concluded that treatment with first-line drugs resulted in suboptimal outcome [8], whereas the other showed that extending the duration of RIF and increasing the number of effective drugs lowered the odds of unfavourable outcome [16]. An analysis of individual patient data conducted in the framework of a World Health Organization (WHO) guideline development process showed that the addition of a fluoroquinolone to a regimen of 6 months of daily RIF, ethambutol (EMB) and pyrazinamide (PZA) was associated with improved treatment success in INH-resistant cases [17]. After an evaluation of all available evidence, WHO has issued new guidelines on treatment for patients with INH mono-resistance [18]. Discussion is ongoing as to whether to maintain INH in the treatment regimen if a low degree of resistance is detected; however, there is limited data on the effect this strategy has on treatment outcome.

The European Surveillance System (TESSy), hosted by the European Centre for Disease Prevention and Control (ECDC), contains case-based information for more than 1.5 million TB cases reported by EU/EEA countries between 1995–2015 [3]. In contrast to the information from randomised controlled trials (RCTs) and cohort studies that were included in the systematic reviews, TESSy includes information on treatment outcome obtained in a programmatic setting [8,16]. These data are from a larger number of patients, are more recent and are more EU/EEA-focused than the data in the aforementioned reviews. We therefore set out to analyse this dataset to determine the current treatment outcome of INH mono-resistant TB in the EU/EEA and to identify risk factors for unsuccessful treatment outcome in cases with INH mono-resistance.

### Methods

#### Study population and data sources

In this observational study, we retrospectively analysed TB notification data reported to the TESSy database between 2002–14. We included pulmonary and extra-pulmonary TB cases with available information on treatment outcome and drug-susceptibility testing (DST) results for at least INH, RIF, streptomycin (STR) and EMB. Information on DST for PZA was not collected in TESSy during the study period and therefore PZA was not part of our inclusion criteria.

#### Operational definitions

Treatment outcome was reported 12, 24 and 36 months after the start of TB treatment. We categorised treatment outcome in accordance with the 2017 joint WHO Regional Office for Europe/ECDC surveillance and monitoring report [3]. For cases with a treatment outcome of ‘still on treatment’ at 12 or 24 months, the final treatment outcome reported at 24 or 36 months was used, respectively. Unadjusted data was stratified by age, although this stratification was not described in the protocol for this study. The operational definitions used in this study are listed in the Box.

#### Statistical analysis and modelling approach

To investigate the impact of INH mono-resistant TB compared with fully drug-susceptible TB on treatment success, we applied a multilevel logistic regression model. To adjust for the heterogeneity between countries, the model was corrected with a random intercept at the country level for the differences in the average treatment success rate between countries and with a random slope for the differences in the INH mono-resistance effect on the treatment success rate at the country level. The necessity of adding a random intercept and a random slope in the model was determined using the likelihood-ratio test. The main outcome was dichotomised as unsuccessful treatment (failed, died, lost to follow-up and not evaluated) vs treatment success (cured or completed), using the final reported treatment outcome. Independent variables available in the TESSy data (age, sex, geographical origin, type of TB, microscopic confirmation, history of TB, HIV status and reporting year) were also assessed as possible

---

**Box**

**Operational definitions for analysis of European tuberculosis surveillance data, 2002–2014**

The following definitions were used:

- **INH mono-resistant TB cases**: cases with resistance to INH and documented susceptibility to Rif, STR and EMB;
- **Cases with fully drug-susceptible TB**: cases with documented susceptibility to INH, Rif, STR and EMB;
- **New TB cases**: cases who were never previously treated for TB or who received drug treatment for less than 1 month;
- **Cases with a history of TB**: cases who were previously treated for TB for 1 month or more (for countries who did not report information about previous treatment, the variable previous diagnosis was used as a proxy);
- **Geographical origin of cases**, i.e. native vs foreign: based on the country of birth or, if this information was unavailable, on the citizenship of the patient;
- **Low TB-incidence country**: country with a TB incidence rate < 10 cases per 100,000 population [31];
- **High TB-incidence country**: country with a TB incidence rate ≥ 10 cases per 100,000 population [31].

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EMB: ethambutol; INH: isoniazid; Rif: rifampicin; STR: streptomycin; TB: tuberculosis.
Figure 1
Flow chart of cases included in analysis of treatment outcome of isoniazid mono-resistant tuberculosis, 31 EU/EEA countries, 2002–2014

1,008,818 TB cases reported to TESSy between 2002 and 2014

164,625 TB cases from countries that do not report treatment outcomes

844,193 TB cases with information on treatment outcome

4,031 TB cases diagnosed after death (post-mortem)

840,162 ante-mortem TB cases with information on treatment outcome

618,291 TB cases without information on DST for INH, RIF, EMB or STR

221,871 ante-mortem TB cases with information on treatment outcome and DST for at least INH, RIF, EMB and STR

809 RIF mono-resistant TB cases
401 EMB mono-resistant TB cases
6,569 STR mono-resistant TB cases

194,948 TB cases included in our study

809 RIF mono-resistant TB cases
401 EMB mono-resistant TB cases
6,569 STR mono-resistant TB cases

12,270 MDR-TB cases
6,874 poly-resistance TB cases

187,370 (96.1%) fully susceptible TB cases
7,578 (3.9%) INH mono-resistant TB cases


According to WHO, poly-resistance in TB cases refers to resistance to two or more first-line drugs, but not to both isoniazid and rifampicin, i.e. not MDR TB.

Data source: TESSy.
<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Excluded TB cases</th>
<th>Included TB cases</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>813,870</td>
<td>194,948</td>
<td>0.05</td>
</tr>
<tr>
<td>Median age in years (IQR)</td>
<td>43 (30–58)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Male sex</td>
<td>522,843</td>
<td>127,642</td>
<td>0.91</td>
</tr>
<tr>
<td>Cases of foreign origin</td>
<td>172,776</td>
<td>56,451</td>
<td>0.51</td>
</tr>
<tr>
<td>Extra-pulmonary TB</td>
<td>177,592</td>
<td>28,032</td>
<td>0.11</td>
</tr>
<tr>
<td>New TB cases</td>
<td>638,026</td>
<td>163,546</td>
<td>0.02</td>
</tr>
<tr>
<td>Microscopic confirmation</td>
<td>296,127</td>
<td>93,560</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Positive HIV status</td>
<td>4,976</td>
<td>1,248</td>
<td>0.68</td>
</tr>
</tbody>
</table>

Proportion of cases by reporting countries

<table>
<thead>
<tr>
<th>Country</th>
<th>Excluded TB cases</th>
<th>Included TB cases</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Austria</td>
<td>4,771</td>
<td>5,919</td>
<td>0.01</td>
</tr>
<tr>
<td>Belgium</td>
<td>13,830</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bulgaria</td>
<td>14,542</td>
<td>5,695</td>
<td>0.13</td>
</tr>
<tr>
<td>Croatia</td>
<td>619</td>
<td>972</td>
<td>0.61</td>
</tr>
<tr>
<td>Cyprus</td>
<td>328</td>
<td>244</td>
<td>0.42</td>
</tr>
<tr>
<td>Czech Republic</td>
<td>4,927</td>
<td>5,704</td>
<td>0.53</td>
</tr>
<tr>
<td>Denmark</td>
<td>4,086</td>
<td>843</td>
<td>0.17</td>
</tr>
<tr>
<td>Estonia</td>
<td>2,927</td>
<td>2,826</td>
<td>0.49</td>
</tr>
<tr>
<td>Finland</td>
<td>2,577</td>
<td>1,817</td>
<td>0.41</td>
</tr>
<tr>
<td>France</td>
<td>70,140</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Germany</td>
<td>28,524</td>
<td>40,015</td>
<td>0.58</td>
</tr>
<tr>
<td>Greece</td>
<td>7,911</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hungary</td>
<td>17,720</td>
<td>4,879</td>
<td>0.21</td>
</tr>
<tr>
<td>Iceland</td>
<td>137</td>
<td>3</td>
<td>0.22</td>
</tr>
<tr>
<td>Ireland</td>
<td>3,660</td>
<td>1,803</td>
<td>0.33</td>
</tr>
<tr>
<td>Italy</td>
<td>56,146</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Latvia</td>
<td>7,533</td>
<td>8,209</td>
<td>0.52</td>
</tr>
<tr>
<td>Liechtenstein</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lithuania</td>
<td>13,572</td>
<td>12,570</td>
<td>0.48</td>
</tr>
<tr>
<td>Luxembourg</td>
<td>443</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Malta</td>
<td>305</td>
<td>138</td>
<td>0.31</td>
</tr>
<tr>
<td>Netherlands</td>
<td>8,314</td>
<td>5,769</td>
<td>0.41</td>
</tr>
<tr>
<td>Norway</td>
<td>1,450</td>
<td>2,771</td>
<td>0.66</td>
</tr>
<tr>
<td>Poland</td>
<td>70,917</td>
<td>39,430</td>
<td>0.36</td>
</tr>
<tr>
<td>Portugal</td>
<td>24,906</td>
<td>16,241</td>
<td>0.39</td>
</tr>
<tr>
<td>Romania</td>
<td>311,991</td>
<td>3,265</td>
<td>1.1</td>
</tr>
<tr>
<td>Slovenia</td>
<td>432</td>
<td>2,372</td>
<td>0.84</td>
</tr>
<tr>
<td>Slovakia</td>
<td>4,189</td>
<td>3,783</td>
<td>0.47</td>
</tr>
<tr>
<td>Spain</td>
<td>54,232</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sweden</td>
<td>5,881</td>
<td>1,264</td>
<td>0.17</td>
</tr>
<tr>
<td>UK</td>
<td>76,855</td>
<td>28,416</td>
<td>0.27</td>
</tr>
</tbody>
</table>

EU/EEA: European Union/European Economic Area; HIV: human immunodeficiency virus; IQR: interquartile range; TB: tuberculosis; TESSy: The European Surveillance System; UK: United Kingdom.

* Obtained by a multivariable logistic regression model corrected for clustering within countries.

† Not performing drug susceptibility testing for streptomycin.

‡ Not reporting case-based drug susceptibility data and treatment outcome data.

Data source: TESSy.
Table 2a
Characteristics of tuberculosis cases by isoniazid mono-resistance status, 24 EU/EEA countries, 2002–2014 (n = 194,948)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Fully susceptible TB cases</th>
<th>INH mono-resistant TB cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Total</td>
<td>187,370</td>
<td>NA</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>64,640</td>
<td>34.5</td>
</tr>
<tr>
<td>Male</td>
<td>122,636</td>
<td>65.4</td>
</tr>
<tr>
<td>Unknown</td>
<td>94</td>
<td>0.1</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>46 (32–60)</td>
<td>NA</td>
</tr>
<tr>
<td>Age group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;15</td>
<td>3,160</td>
<td>1.7</td>
</tr>
<tr>
<td>15–44</td>
<td>89,845</td>
<td>47.9</td>
</tr>
<tr>
<td>45–64</td>
<td>57,172</td>
<td>30.5</td>
</tr>
<tr>
<td>&gt;64</td>
<td>37,127</td>
<td>19.8</td>
</tr>
<tr>
<td>Unknown</td>
<td>66</td>
<td>0.1</td>
</tr>
<tr>
<td>Geographical origin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Native</td>
<td>131,344</td>
<td>70.1</td>
</tr>
<tr>
<td>Foreign</td>
<td>53,648</td>
<td>28.6</td>
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<tr>
<td>Unknown</td>
<td>2,378</td>
<td>1.3</td>
</tr>
<tr>
<td>Type of TB</td>
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<td></td>
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<tr>
<td>Pulmonary</td>
<td>160,231</td>
<td>85.5</td>
</tr>
<tr>
<td>Extra-pulmonary</td>
<td>26,813</td>
<td>14.3</td>
</tr>
<tr>
<td>Unknown</td>
<td>326</td>
<td>0.2</td>
</tr>
<tr>
<td>Sputum smear microscopy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>57,063</td>
<td>30.4</td>
</tr>
<tr>
<td>Positive</td>
<td>89,898</td>
<td>47.9</td>
</tr>
<tr>
<td>Unknown</td>
<td>40,409</td>
<td>21.7</td>
</tr>
<tr>
<td>History of TB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>New TB case</td>
<td>157,526</td>
<td>84.1</td>
</tr>
<tr>
<td>Case with history of TB</td>
<td>17,634</td>
<td>9.4</td>
</tr>
<tr>
<td>Case with unknown TB history</td>
<td>12,210</td>
<td>6.5</td>
</tr>
<tr>
<td>HIV status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>17,624</td>
<td>9.4</td>
</tr>
<tr>
<td>Positive</td>
<td>1,203</td>
<td>0.6</td>
</tr>
<tr>
<td>Unknown</td>
<td>168,543</td>
<td>90.0</td>
</tr>
<tr>
<td>EU/EEA countries</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low TB incidence</td>
<td>74,742</td>
<td>40.0</td>
</tr>
<tr>
<td>High TB incidenceb</td>
<td>112,628</td>
<td>60.0</td>
</tr>
<tr>
<td>Reporting years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2002–2005</td>
<td>49,532</td>
<td>26.4</td>
</tr>
<tr>
<td>2006–2009</td>
<td>61,478</td>
<td>32.8</td>
</tr>
<tr>
<td>2010–2014</td>
<td>76,360</td>
<td>40.8</td>
</tr>
</tbody>
</table>

EU/EEA: European Union/European Economic Area; INH: isoniazid; IQR: interquartile range; NA: not applicable; TB: tuberculosis; TESSy: The European Surveillance System; UK: United Kingdom.

* TB cases susceptible to at least isoniazid, rifampicin, ethambutol and streptomycin.

† High-incidence countries were defined as those with 10 or more TB cases per 100,000 population in 2015 (Bulgaria, Croatia, Estonia, Latvia, Lithuania, Poland, Portugal, Romania and the UK).

Data source: TESSy.
confounders in the relationship between INH mono-resistant TB and treatment success. Independent variables that caused a change in the regression coefficient between INH mono-resistant TB and treatment success of >10% were considered potential confounders and were retained in the final multilevel multivariable model. In addition, we evaluated the interaction of INH mono-resistant TB with age and history of TB on treatment success at a p value of 0.1. Furthermore, a sensitivity analysis was conducted to assess the impact of INH mono-resistance after excluding cases with the outcome ‘not evaluated’.

To identify risk factors for an unsuccessful treatment outcome in INH mono-resistant cases, the multilevel univariate and multivariable logistic regression models were used to examine the association between predicting variables (age, sex, geographical origin, microscopy confirmation, history of TB, HIV status, type of TB and reporting year) and unsuccessful treatment outcome. Age was dichotomised into < and ≥ the median age of

### Table 2b

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Fully susceptible TB cases&lt;sup&gt;a&lt;/sup&gt;</th>
<th>INH mono-resistant TB cases</th>
<th>n</th>
<th>%</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of TB cases by reporting country</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
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<td>Austria</td>
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<td>58</td>
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<td>78</td>
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<tr>
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<td>94.1</td>
<td>1,691</td>
<td>5.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

EU/EEA: European Union/European Economic Area; INH: isoniazid; TB: tuberculosis; TESSy: The European Surveillance System; UK: United Kingdom.

<sup>a</sup> TB cases susceptible to at least isoniazid, rifampicin, ethambutol and streptomycin.

<sup>b</sup> Seven countries (Belgium, France, Greece, Italy, Liechtenstein, Luxembourg and Spain) were excluded from this study, as they did not report treatment outcome and/or the required susceptibility data.

Data source: TESSy.
In a sensitivity analysis, cases with the outcome ‘not evaluated’ were excluded. As the outcomes were proportions, we used logistic regression for all models. Odds ratios (OR) with 95% confidence intervals (CI) were calculated to assess the strength of the association. All analyses were performed using the STATA (Stata/SE 14.1, StataCorp LP, Texas, United States (US)) software.

**Ethical statement**
This study is based on data collected on the basis of statutory notification in each EU/EEA country and reported anonymously to ECDC on the basis of decision No 2119/98/EC of the European Parliament and of the Council [19]. Therefore, informed consent from patients is not required.

**Results**

**Characteristics of the study population**
From 2002–14, a total of 1,008,818 TB cases were notified in 31 EU/EEA countries and reported to TESSy. TB cases without reported treatment outcome for the years 2002–14 (n = 164,625) or who were diagnosed post-mortem (n = 4,031) were excluded from our analysis. We also excluded 618,291 TB cases without DST results for INH, RIF, EMB or STR. Furthermore, we excluded cases with drug resistance other than INH mono-resistance (n = 26,923). The remaining 194,948 TB cases from 24 EU/EEA: European Union/European Economic Area; INH: isoniazid; TESSy: The European Surveillance System.

\*a Treatment outcome at 12 months.
\*b Final reported treatment outcome.

Data source: TESSy.
Impact of INH mono-resistance on tuberculosis treatment success

In the univariate model corrected for clustering within countries, INH mono-resistance was associated with a lower TB treatment success compared with cases with fully susceptible TB (OR: 0.8; 95% CI: 0.7–0.9). Out of all statistically evaluated covariates, adding age, microscopic confirmation or history of TB to the crude model led to the predefined change (10%) in the regression coefficient for INH mono-resistance and, therefore, these covariates were retained in the multivariable model as potential confounders. No interactions of INH mono-resistant TB with age (p value = 0.9) or history of TB treatment (p value = 0.2) were observed; therefore, these variables were not included in the final model.

In the final multivariable model, treatment success among INH mono-resistant TB was lower compared with fully drug-susceptible TB (adjusted OR: 0.7; 95% CI: 0.6–0.9). This corresponds to an adjusted treatment success of 74.0% for INH mono-resistant TB and 79.3% for fully susceptible TB, adjusted absolute difference of 5.3% (Supplementary Table S1). The treatment success remained lower among INH mono-resistant TB compared with fully susceptible TB in the multivariable model after excluding cases with ‘not evaluated’ as treatment outcome (OR: 0.7; 95% CI: 0.6–0.8) (data not shown).

Factors associated with unsuccessful final treatment outcome of INH mono-resistant tuberculosis cases

In the multivariable model adjusted for heterogeneity between countries, unsuccessful treatment among INH mono-resistant TB cases was associated with age ≥ median age (41 years) (OR: 1.3; 95% CI: 1.2–1.5), male sex (OR: 1.3 95%; CI: 1.1–1.4), positive microscopy (OR: 1.3; 95% CI: 1.1–1.4), history of TB (OR: 1.8; 95% CI: 1.5–2.2) and positive HIV status (OR: 3.3; 95% CI: 1.6–6.5) (Figure 4) (Supplementary Table S2). In the sensitivity analysis, excluding cases with the treatment outcome ‘not evaluated’, no change in the associated risk factors was observed (data not shown).

INH mono-resistant and fully susceptible TB shared the same risk factors for unsuccessful treatment, except for being of foreign origin, which was associated with a higher risk for unsuccessful treatment in fully susceptible but not INH mono-resistant TB cases in the multivariable model (data not shown).

Discussion

Our retrospective study of European surveillance data, including 7,578 cases of INH mono-resistant TB and 187,370 cases of fully susceptible TB, shows that INH mono-resistance is associated with lower TB treatment success in the final TB treatment outcome. This association between INH mono-resistance and lower treatment success is in line with a previous systematic review [20]. Although this review was published in 2009, it includes many studies that were published...
Figure 3
Treatment outcome of tuberculosis by isoniazid mono-resistance status and (A) age group and (B) reporting country, 24 EU/EEA countries, 2002–2014 (n = 194,948)

A.

![Graph showing treatment outcome of tuberculosis by isoniazid mono-resistance status and age group](image)

B.

![Graph showing treatment outcome of tuberculosis by isoniazid mono-resistance status and reporting country](image)


* Information missing for 67 cases.

Data source: TESSy.
before the year 2000 and were mainly conducted in countries in Asia and Africa. The same applies to the reviews evaluating treatment regimens for INH mono-resistant TB that were published in 2016 [16] and 2017 [8], respectively. Therefore, our analysis of more recent 2002–14 EU/EEA surveillance data represents an addition to the currently available evidence.

Studies of TB treatment outcome under routine programmatic conditions have shown inconsistent results regarding treatment outcome of INH mono-resistant TB. While studies from the US [10], Denmark [12] and Israel [21] reported treatment outcome for INH mono-resistant TB as excellent, highly successful or similar to drug-susceptible TB, studies from Peru [22], Mexico [23], Georgia [24] and South Africa [11] showed poorer treatment outcome compared with fully susceptible TB. Possible explanations might be differences in the included patient populations; for example, regarding HIV prevalence [11,12], the availability of resources for patients, the accessibility of healthcare systems or the use of different treatment regimens. Our study, which pools data from 24 low HIV-prevalence and predominantly high-income European countries, shows that INH mono-resistance negatively affects treatment outcome. The study findings also highlight the need for timely identification of patients with INH mono-resistant TB, especially as rapid testing in recent years has focused more on the detection of RIF resistance as a proxy for MDR TB [6].

CI: confidence interval; EU/EEA: European Union/European Economic Area; INH: Isoniazid; TB: tuberculosis; TESSy: the European Surveillance System.

Univariable and multivariable analyses were based on multilevel logistic regression models corrected for clustering within countries using an unstructured covariance matrix. TB cases with available information for all predicting factors were included in the multivariable analysis (n = 5,759/7,578). Excluded cases have a slightly higher treatment success (74.7% vs 73.8%; p value = 0.46).

Data source: TESSy.
Comparison of studies has been hampered by different definitions used for INH mono-resistance. While many studies retained all INH resistance profiles, provided that RIF resistance and therefore MDR TB was excluded [8,16], other studies required additional documented susceptibility for STR and EMB [23] or analysed INH mono-resistant and INH poly-resistant cases with additional resistance to STR or EMB separately [12]. To avoid misclassification, we have used the stricter definition requiring documented DST results for INH, RIF, STR and EMB for all cases. However, while gaining specificity in the definition of INH mono-resistance, this approach has resulted in the loss of a large number of cases for which these susceptibility testing results were not available, with considerable differences in the percentages of cases that could be included by country.

The finding that EU/EEA countries have different proportions of patients with INH mono-resistant TB still under treatment at 12 months might be a surveillance artefact related to variation in reporting procedures or the result of the use of different treatment regimens with different durations, reflecting the current lack of an agreed standard regimen for the treatment of INH mono-resistant TB cases [18,25,26]. As the 12-month outcome therefore did not seem to be the most adequate endpoint for analysis of the treatment outcome of INH mono-resistant TB, we chose a composite outcome for this study using the final documented outcome irrespective of the time to reporting (12 months, 24 months or 36 months after the start of treatment).

The factors found to be associated with a higher risk of unsuccessful treatment outcome in INH mono-resistant TB in this study—higher age, male sex, positive microscopy, positive HIV status—have been described before as associated with unsuccessful TB treatment outcome independent of drug resistance status [27,28]. Prior TB treatment has also been reported as a risk factor for unsuccessful outcome in patients with INH mono-resistant TB [29]. In our study, these factors influenced TB treatment outcome regardless of the presence or absence of INH mono-resistance, indicating that they are not specific to INH mono-resistant TB, but rather are associated with lower TB treatment success in general.

The strengths of our study are the inclusion of a large number of cases and the application of a multilevel model to correct for TB clustering. This allowed us to control for possible selection bias related to reporting countries and for unobserved heterogeneity between countries, thereby enhancing the generalisability of our findings. However, there are also several limitations to our study, mainly related to the use of surveillance data collected with the aim to inform TB programme management and not to evaluate clinical outcomes. As a result, data on the severity of TB disease, underlying diseases and treatment regimens were not available.

In addition, information on DST for PZA is not available in the TESSy data. In a sensitivity analysis using German notification data (40,063 TB cases, of which 1,310 were INH mono-resistant) that include information on DST for PZA, cases resistant to PZA were more frequent among INH mono-resistant TB compared with otherwise fully susceptible TB cases (5.4% vs 1.8%; p<0.01). However, no impact of the PZA resistance status on the relationship between INH mono-resistant TB and treatment success was observed (adjusted OR: 0.8; 95% CI: 0.6–1.4; p=0.77). Another limitation related to DST is that no information is available on the level of INH-resistance and the type of INH resistance mutations involved, which have been shown to influence treatment outcome [30].

Lastly, our study included data from only 24 of 31 EU/EEA countries; cases from seven countries were excluded due to lack of reporting treatment outcome or case-based susceptibility data (Table 1). Therefore, our data pertain only to these 24 EU/EEA countries and cannot be generalised to the whole EU/EEA without caution. Of note, 80.7% of reported cases had to be excluded due to missing information. As systematic reasons for lack of reporting within the 24 countries with included cases are not known, it is not possible for us to hypothesise how this might have affected our findings.

In conclusion, this study shows that treatment of patients with INH mono-resistant TB under routine programme conditions leads to lower treatment success compared with fully susceptible TB. The association of INH mono-resistance with negative treatment outcome highlights the need to pay increased attention to the timely identification and management of these cases to ensure treatment success for individual patients, as well as to reduce the risk for further resistance development on a population level.

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Any supplementary material referenced in the article can be found in the online version.

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Visual tools to assess the plausibility of algorithm-identified infectious disease clusters: an application to mumps data from the Netherlands dating from January 2009 to June 2016

Loes Soetens1,2, Jantien A. Backer1, Susan Hahné1, Rob van Binnendijk1, Sigrid Gouma1,3, Jacco Wallinga1,2
1. Centre for Infectious Disease Control, National Institute for Public Health and the Environment, Bilthoven, The Netherlands
2. Medical Statistics, Department of Biomedical Data Sciences, Leiden University Medical Center, Leiden, The Netherlands
3. Department of Microbiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, USA

Correspondence: Loes Soetens (loes.soetens@rivm.nl)

Introduction: With growing amounts of data available, identification of clusters of persons linked to each other by transmission of an infectious disease increasingly relies on automated algorithms. We propose cluster finding to be a two-step process: first, possible transmission clusters are identified using a cluster algorithm, second, the plausibility that the identified clusters represent genuine transmission clusters is evaluated. Aim: To introduce visual tools to assess automatically identified clusters. Methods: We developed tools to visualise: (i) clusters found in dimensions of time, geographical location and genetic data; (ii) nested sub-clusters within identified clusters; (iii) intra-cluster pairwise dissimilarities per dimension; (iv) intra-cluster correlation between dimensions. We applied our tools to notified mumps cases in the Netherlands with available disease onset date (January 2009 – June 2016), geographical information (location of residence), and pathogen sequence data (n = 112). We compared identified clusters to clusters reported by the Netherlands Early Warning Committee (NEWC). Results: We identified five mumps clusters. Three clusters were considered plausible. One was questionable because, in phylogenetic analysis, genetic sequences related to it segregated in two groups. One was implausible with no smaller nested clusters, high intra-cluster dissimilarities on all dimensions, and low intra-cluster correlation between dimensions. The NEWC reports concurred with our findings: the plausible/questionable clusters corresponded to reported outbreaks; the implausible cluster did not. Conclusion: Our tools for assessing automatically identified clusters allow outbreak investigators to rapidly spot plausible transmission clusters for mumps and other human-to-human transmissible diseases. This fast information processing potentially reduces workload.

Introduction

Individual case data originating from routine infectious disease surveillance more and more also include genetic sequence information. With increasing availability of different types of data (e.g. geographical data, time, genetic sequence), each adding their own dimension, and quantities of data rising, transmission-cluster identification of infectious diseases progressively relies on automated algorithms. A transmission cluster can be defined as several cases of an infectious disease which are connected by transmission of this disease from one person to another. A transmission chain is then defined as a series of cases connected by transmission events. Much work has been done on developing algorithms to identify transmission clusters of cases using large datasets [1]. Existing algorithms focus on cluster identification in time [2-9], in space or space-time [10-12], in genetics [13-15], or by combining all three data dimensions [16-18].

A major challenge with clustering algorithms is to balance specificity and sensitivity. If an algorithm lacks specificity, it finds clusters of cases even though there are no transmission events that link them. If it lacks sensitivity, the algorithm does not find genuine transmission chains. To be on the safe side, most algorithms have a high sensitivity at the expense of specificity and as a result also identify clusters of cases that are not genuine transmission clusters. We therefore propose cluster detection using algorithms as a two-step process: (i) detecting possible clusters of infectious diseases with an algorithm and (ii) assessing the plausibility that an identified cluster represents a transmission cluster.
While there has been much work on the first step, little research attention has been paid to methods for improving the plausibility assessment. Currently, identified clusters are usually assessed by epidemiologists who assess information and verify it through communicating with the municipal health services (MHS). This can be quite labor intensive, especially if there are many identified clusters stretching across multiple regions. Only recently, a study has been published that introduced a framework for computing epidemiological concordance of microbial subtyping data of *Campylobacter jejuni* [19]. Epidemiological cluster cohesion is based on time, geographical location, and environmental source distances with adjustable weights. This method requires the computation of a disease specific source distance matrix, making it difficult to apply generically. To our knowledge no further tools are available for careful plausibility assessment of automatically detected clusters.

In order to develop such tools, general characteristics for discriminating transmission clusters from non-transmission clusters have to be identified. We propose to assess the variation of clusters in their time, geographical location and genetic profile. The variation on these dimensions can be visualised by projecting cases on an epidemic curve, map, and phylogenetic tree, respectively, as well as by estimating the relative distance between clustered cases on these respective dimensions and comparing the distance to the inter-case distances from non-clustered cases. It is assumed that clustered cases will have smaller inter-case distances on these respective dimensions than non-clustered cases. However, there are exceptions: an outbreak may show large variation in time between the occurrence of cases (single persistent source, e.g. typhoid [20]), large variation in geographical distances between cases (initial cases travel large distances, e.g. severe acute respiratory syndrome (SARS) [21]), or include large genetic sequence variation in the pathogen causing the outbreak (fast mutating strains, e.g. Ebola [22,23]). In order to settle several of these exceptions, intra-cluster correlation between the data dimensions time, geographical location and genetics can be used as another discriminatory characteristic. In genuine transmission clusters, variation on one

<table>
<thead>
<tr>
<th>No</th>
<th>Date reported</th>
<th>Reported by</th>
<th>Covering time period</th>
<th>Number of cases in report</th>
<th>Age range (years)</th>
<th>Remark/source</th>
<th>Cluster number according to current study</th>
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<td>RIVM</td>
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<td>171</td>
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<td>Start of nationwide mumps epidemic</td>
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<td>12 Feb</td>
<td>RIVM</td>
<td>Dec 2009–Feb 2012</td>
<td>1,264</td>
<td>NR</td>
<td>Overview of nationwide mumps epidemic</td>
<td>NL</td>
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<td>3</td>
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<td>15–26</td>
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<td>4</td>
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<td>Jul 2012</td>
<td>3</td>
<td>6–8</td>
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<td>Jun 2013</td>
<td>11</td>
<td>23–29</td>
<td>Unknown</td>
<td>NL</td>
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<td>13 Nov</td>
<td>GGD Zaanstreek - Waterland</td>
<td>Sep 2013–Nov 2013</td>
<td>16</td>
<td>4–47</td>
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<td>9</td>
<td>13 Nov</td>
<td>GGD Groningen</td>
<td>Sep 2013–Nov 2013</td>
<td>13</td>
<td>17–36</td>
<td>Students</td>
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<td>14 Feb</td>
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<td>3</td>
<td>25–30</td>
<td>Work in healthcare setting</td>
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<td>11</td>
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<td>5</td>
<td>NR</td>
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<tr>
<td>12</td>
<td>15 Jun</td>
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<td>Apr 2015–Jun 2015</td>
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<td>NR</td>
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<td>16 Mar</td>
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<td>16 Apr</td>
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<td>Mar 2016–Apr 2016</td>
<td>6</td>
<td>17–23</td>
<td>Friends/party</td>
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GGD: Gemeentelijke GezondheidsDienst (Municipal Health Service); NL: no link to a cluster; NR: not reported; RIVM: Rijksinstituut voor Volksgezondheid en Milieu (National institute for public health and the environment).
dimension tends to be correlated with the other dimension. For example, with tuberculosis, cases within a genuine transmission cluster with a larger genetic distance, also tend to have a larger distance in time [24].

The largest hurdle to effectively use algorithms in outbreak investigations is the interpretation of their output, rather than the application of the algorithms themselves. As visualisation techniques support fast processing of large amounts of information, developing tools for visually assessing the plausibility of transmission clusters identified through statistical algorithms may help outbreak investigators [25]. Moreover if data are available in a timely fashion, this may allow pointing outbreak investigators to the most plausible signals first, which, when time is scarce, may facilitate task prioritisation. Finally, outbreak information, such as what is obtained with various available tools (e.g. typical cluster size, typical inter-case distance and correlate estimates between dimensions for a specific disease), might contribute to our current understanding of transmission model parameters [26,27].

To apply and assess the tools that we develop, we use mumps notification and sequence data reported between 2009 and mid-2016 in the Netherlands. We specifically chose mumps in the Netherlands as it has been intensively studied over the past few years, with comprehensive documentation available [28-33]. In the Netherlands, mumps is a notifiable disease and symptomatic cases are reported to the MHS by physicians and/or laboratories. Cases are either notified when there is a laboratory confirmation or when there is an established epidemiological link with a confirmed case. In case of laboratory confirmation, the national reference laboratory aims to obtain material from regional laboratories for further sequencing. Sequencing provides information on the circulating genotypes and

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**Figure 1**
Hierarchical clustering tree of the combined dissimilarities of all dimensions for cases of mumps in the Netherlands, January 2009–May 2016 (n = 112 cases)

- Unclustered cases, n = 47 cases
- Cluster 1, n = 3 cases
- Cluster 2, n = 9 cases
- Cluster 3, n = 12 cases
- Cluster 4, n = 13 cases
- Cluster 5, n = 28 cases

All dimensions include time, geographical location or genetic dimensions.

The colours represent the cases belonging to significant highest unnested clusters. The black dots represent significant clusters (p < 0.001) at all nesting levels.

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Figure 2
Identified clusters of mumps with cases projected on (a) an epicurve (time), (b) maps of the Netherlands (geographical location) and (c) an arbitrarily rooted maximum likelihood phylogenetic tree of the pathogen sequences (genetics), Netherlands, January 2009–May 2016 (n = 112 cases)

A.

B.

C.

The colours indicate the significant highest unnested clusters identified with the time-place-type algorithm (cluster 1 is red, 2 blue, 3 green, 4 pink, 5 orange); the unclustered cases are depicted in grey. The scale represents the number of nucleotide substitutions per site.
helps to assess whether there is endemic circulation or new introductions of mumps viruses in the country.

Currently, epidemiologists mainly rely on epidemic curves (time data dimension) to detect mumps outbreaks. We set out to assess whether combining geographical location, time and genetic information can contribute to mumps cluster identification. We use an existing clustering algorithm which can take into account these three data dimensions [16,17] (hereafter: the time-place-type clustering algorithm), and which is already in use to identify outbreaks of various diseases in the Netherlands, such as meningococcal W disease, meticillin-resistant *Staphyloccocus aureus* (MRSA) [17] and echovirus type 6 [34]. We develop and validate visual tools to determine whether identified clusters with this algorithm represent transmission clusters.

**Methods**

**Data**

In this study, we include all notified mumps cases in the Netherlands who were diagnosed between 1 January 2009 and 31 May 2016. Notification criteria for mumps include more than one related symptom (i.e. acute onset of painful swelling of the parotid or other salivary glands, orchitis, or meningitis) and laboratory confirmation of infection or an epidemiologic link to a laboratory-confirmed case. The notification criteria did not change during the study period.

For our analysis, for each case we require data on three factors. The first is the disease onset date, which is collected during routine surveillance. The second is the geographical location. The geographical location can be any location that is most relevant for the transmission pattern of the disease under study. For pragmatic reasons, this is usually the location of residence of the case, but this might also be a working address or other place visited. In this study, we used more specifically the latitude and longitude of location of residence of the cases. In the Netherlands, cases’ four digit postal code of residence is collected during routine surveillance. The second is the disease onset date, which is collected during routine surveillance. The second is the disease onset date, which is collected during routine surveillance. The third required factor is the code of residence is collected during routine surveillance. The second is the disease onset date, which is collected during routine surveillance. The third required factor is the disease onset date, which is collected during routine surveillance. The third required factor is the geographical location. The geographical location can be any location that is most relevant for the transmission pattern of the disease under study. For pragmatic reasons, this is usually the location of residence of the case, but this might also be a working address or other place visited. In this study, we used more specifically the latitude and longitude of location of residence of the cases. In the Netherlands, cases’ four digit postal code of residence is collected during routine surveillance. We take the centroids of the four digit postal codes and use its latitude and longitude as the input variables for the algorithm. The third required factor consists of the sequences of the small hydrophobic (SH) gene (316 bp), the haemagglutinin/neuraminidase (HN) gene (1,749 bp), and the fusion (F) gene (1,617 bp). Sequences of these three genes are used in combination to distinguish between different mumps genotypes [35], and clusters within genotype G [33]. Since the algorithm that we use is only able to handle cases with data on all three dimensions, cases with missing data on one of the three required factors are excluded from our analysis.

**Cluster algorithm**

In order to find infectious disease transmission clusters using time, geographical location, and genetic information, Ypma et al. [16] have developed an algorithm to combine pairwise distances between cases on all three data dimensions into one metric. The algorithm sorts cases by relatedness on all three dimensions and subsequently defines a relative distance for all possible pairs of cases reflecting the number of cases found in between the two cases. The relative distances (dissimilarities) for each dimension are calculated, and the combined dissimilarity ($d_{comb}$) between every pair of cases is then defined as the product of the separate dimension dissimilarities. Next, the cases are joined to form a hierarchical tree of related cases, based on $d_{comb}$, using single-linkage clustering. For every cluster in the tree, statistical significance of each cluster given its height and cluster size is calculated using permutation. More details on the algorithm are presented in Supplement S1.

To demonstrate the tools in this paper, we choose a p value < 0.001 as cut-off level for significance of clusters and consider only the clusters that are not nested within other identified clusters (hereafter ‘highest unnested clusters’) at that cut-off level. Since the p value cut-off level is an arbitrary choice, we add flexibility to the tool, by allowing setting cut-offs for p value, maximum tree-height and maximum cluster size.

**Assessing the plausibility of clusters representing transmission events**

We have developed four tools to assess the plausibility that clusters identified by the time-place-type clustering algorithm represent transmission events. Below we describe each of these four tools in detail.

**Overview visualisation of the clusters in the time, geographical location and genetic dimensions**

To assess the variation in time, geographical location and genetic profile of the identified clusters, we visualise the distribution over time by projecting clusters on an epidemic curve (a histogram showing the distribution of cases over time). The distribution across geographical location is visualised by projecting cases coloured according to their cluster membership on a proportional symbol map, in which the point size is proportional to the number of cases at that location. In the interactive version of the tool, this map is replaced by an interactive dot map, which allows for zooming. The distribution across the genetic dimension is visualised by projecting clusters on an arbitrarily rooted maximum likelihood phylogenetic tree. Only the significant highest unnested clusters are visualised, using different colours for every cluster.

**Hierarchical clustering tree to visualise the nesting of clusters**

Identified clusters can be nested within larger clusters. The structure of the nesting provides valuable information on the strength of the clusters, for example, a cluster that contains several significant clusters at a lower nesting level is stronger than a cluster with no significant clusters at a lower nesting level. Therefore, the structure of the nesting is visualised by providing a
hierarchical clustering tree of related cases, a dendrogram, based on \( d_{\text{combi}} \). The significant highest unnested clusters are visualised by colouring the end-nodes, and all significant clusters are visualised using black dots at the significant internal nodes.

**Intra-cluster pairwise dissimilarity per dimension**

To determine the impact of each dimension on \( d_{\text{combi}} \) (time, geographical location or genetics), we calculate for every significant highest unnested cluster the pairwise dissimilarities per dimension (time, geographical location, genetics, and combined). The pairwise dissimilarities are a measure for intra-cluster variance for the different dimensions. The median dissimilarity is defined as the median of the pairwise dissimilarities \( (d_{\text{time}}, d_{\text{geo}}, d_{\text{gen}}, \text{and} \ d_{\text{combi}}) \) per cluster. We visualise these pairwise dissimilarities using notched boxplots [36]. In a notched box plot, the notches extend 1.58 \times \text{interquartile range (IQR)} / \sqrt{n}, \text{which roughly corresponds to a 95% tolerance interval (assuming a normal distribution). The notches are then used to compare medians, i.e. non-overlapping notches for two different dimensions suggest that the medians are significantly different.}

**Intra-cluster correlations between the different dimensions**

We visualise the intra-cluster correlation of the pairwise dissimilarities between the different dimensions. The intra-cluster correlation provides information on the internal cohesion of a significant cluster, for example, if cases within a cluster are close in time (small \( d_{\text{time}} \)), are also close in geographical space (small \( d_{\text{geo}} \)). In addition, the intra-cluster correlation coefficient between the separate dimensions and the combined dimension informs us on the contribution of each dimension to
the combined dimension. For every significant highest unnested cluster, we compute the Spearman rank correlation coefficient \( r \) between the pairwise dissimilarities of all dimensions and its p-value [37]. We visualise the strength and direction of the correlation coefficients per cluster using a matrix layout. In the interactive version of the tool, one can hover over the matrices to allow for the correlation coefficients and p-values to pop up.

Epidemiological validation

We use epidemiological information to check the validity of the identified significant highest unnested clusters. The gold standard for confirming transmission links is the presence of an epidemiological link between cases. However, this information is only available for a very small subset of mumps cases and is only described in free text fields, which is difficult to analyse. We therefore use mumps outbreaks described in the reports of the Netherlands Early Warning Committee (NEWC) as gold-standard-identified clusters and assess whether these outbreaks correspond to clusters identified with the algorithm [16]. The clusters that do not correspond with the reported outbreaks are considered false positives. In addition, we check whether the identified clusters are described in the literature.

Analysis with only two dimensions

Since the algorithm cannot handle missing data and since genetic data are often delayed or missing, in Supplement S2 we show results when using time and geographical location data only.

Availability

All analyses are performed in R 3.4.3 [38]. We have developed an R package ClusterViz containing an R shiny app to allow users to interactively set parameter values such as cut-offs for p values, tree heights, and cluster sizes. The R package can be downloaded from a github page (https://github.com/lsoetens/ClusterViz). A demo file for testing the tool is also available with this package (as described below). Considering the genetic data used in this study, all F gene, SH gene and HN gene sequences are submitted to the GenBank database and are available with the accession numbers KJ125045–51, KJ125053–9, KJ125061–7, and KU756625-930.

Ethical statement

Due to privacy concerns, data on date of diagnosis and geographical location are not published in any public database. Thus we have slightly obfuscated the time and geographical location data and have added the data file as a demo file to the R package.

In accordance with Dutch law, no informed consent was required for this study using anonymised routine surveillance data.

Results

Between 1 January 2009 and 30 June 2016, 2,039 cases of mumps were reported in the Netherlands. A sequenced sample of the SH, HN, and F gene was available for 118 (5.8%) of the cases. Of the 118 cases with sequenced data, six had missing geographical data. Therefore, 112 (5.5%) cases were included in the analysis. These cases were mainly male (n = 65; 58.0%) and had a median age of 24 years (IQR: 20–27 years). In this study period, 14 mumps related signals were reported by the NEWC (Table 1).

Figures 1 to 4 represent output from our tool. The algorithm identifies 10 clusters with \( p \leq 0.001 \) of which five are nested (Figure 1). After collapsing the nested clusters into their parent clusters, five significant highest unnested clusters remain. Of those five highest unnested clusters clusters, cluster 2 (blue, n = 9), 3 (green, n = 12) and 4 (pink, n = 13) contain smaller clusters which are also significant, whereas cluster 1 (red, n = 3) and 5 (orange, n = 28) are not supported by other significant clusters at a lower nesting level.

To assess the plausibility of the clusters for a specific disease, we focus on the variation within clusters across the time, geographical location or genetic dimension. The clusters show differences in how the cases and samples are distributed over time (Figure 2a), geographical location (Figure 2b), and sequence space (Figure 2c). Compared with clusters 4 and 5, cluster 1, 2 and 3 are very compact on all three dimensions (time, geographical location, and genetics). While cluster 4 is relatively concentrated in time and geographical location, it is distributed across two branches of the phylogenetic tree. For mumps this makes it less plausible that all cases belong to the same transmission chain, as the mumps virus is characterised by a very low mutation rate [39]. For each of the two clusters nested within cluster 4 in the hierarchical tree, cases are located on two branches of the phylogenetic tree, suggesting that also the nested clusters contain substantial genetic disparity. Cluster 5 is quite dispersed on all three dimensions (time, geographical location, and genetics), making this cluster very implausible.

We estimate and visualise for every significant highest unnested cluster the pairwise dissimilarities per data dimension (Figure 3). We find that the median pairwise dissimilarity is significantly lower on the combined dimension in all clusters when compared with the combined pairwise dissimilarity in the unclustered cases. Of the five clusters, cluster 1 has the lowest median pairwise dissimilarities on the three individual dimensions and their combination and cluster 5 has the highest intra-cluster variance on the three individual dimensions and their combination.

We visualise the intra-cluster Spearman rank correlation coefficient \( r \) of the pairwise dissimilarities between the different dimensions (Figure 4). When looking at the correlation coefficients between the data
dimensions time, geographical location and genetics, we can see that many correlation coefficients either cannot be estimated due to zero variance (identical sequences) on the genetics dimension (cluster 1 and 2) or are not statistically significant (p > 0.05). Only in cluster 3 the time dimension is significantly correlated with the geographical location ($r = 0.4$) and genetics ($r = 0.5$) data dimension, and in cluster 4 and 5 the time dimension is correlated with the genetics dimension only ($r = 0.3$ and $r = 0.2$ respectively). When then looking at the contribution of the individual data dimensions to the combined dimension, we can see that in cluster 1, 2 and 3, the dimension of time and geographical location contribute equally and strongly to the combined dimension ($r = (0.9, 0.6, 0.9)$), and in cluster 4 and 5 the dimension of genetics contributes the most information to the combined dimension ($r = (0.8, 0.7)$).

As a measure of validity, we have assessed whether mumps outbreaks described in the reports of the NEWC correspond to clusters identified with the time-place-type algorithm. Clusters 1–4 are easily linkable to reported mumps outbreaks of the NEWC (Table 1). Given its time, period, and the spatial distribution, cluster 1 corresponds to outbreaks 13 and 14, cluster 2 corresponds to outbreaks 11 and 12, cluster 3 corresponds to outbreak 8, and cluster 4 corresponds to outbreak 1. Cluster 5 is the only identified cluster to which no clear reported outbreaks can be linked. Outbreaks 3, 5, and 6 might together possibly compose cluster 5. In addition to the NEWC reports, clusters 2 and 3 are described in references [33] and [32], respectively.

Finally, analysis using time and geographical location data only (Supplement S2) shows that our visual plausibility tools can also be used when data are only available for two dimensions. The cases included in the main analysis are representative for the total notified mumps cases from 2013 onwards, as the shape of the epidemic curves is comparable. However, before 2013 the shapes of the epicurves differ: in the main analysis the large peaks in 2010, 2011 and 2012 cannot be observed. In 2013–16, we identify six clusters using only two dimensions that are similar to those identified using three dimensions, we miss only three minor clusters. In the period before 2013, nine additional clusters are identified in the time-place analysis, of which three are very large (n > 40). The lesser plausible pink (cluster 4) and orange (cluster 5) clusters from the main analysis fall in the less representative period before 2013, so it might be due to unrepresentative sequencing in this period that transmission cluster detection with this algorithm is more difficult.

**Discussion**

In this paper, we have introduced tools in order to assess the plausibility of transmission clusters. In the mumps case study, five significant clusters are identified, several of which also contain nested clusters. In assessing the plausibility of these significant clusters, the tools that we have developed all point in the same direction: clusters 1 (red), 2 (blue), and 3 (green) can be considered highly plausible; cluster 4 (pink) has moderate plausibility as the sequences related to it span across two branches of the phylogenetic tree; and cluster 5 (orange) has low plausibility. Compared with the other clusters, cluster 5 shows a relatively dispersed pattern across time, geographical location, and genetics; contains no nested clusters; shows relatively high intra-cluster dissimilarity on all dimensions; and shows the lowest intra-cluster correlation between all four dimensions. In our epidemiological validation, no clear reported outbreak can be linked to cluster 5. In contrast, the other four identified clusters are easily linkable to a reported outbreak.

The major advantage of our tools is that we use visualisation techniques to improve assessment of plausibility. Human vision supports fast processing of
information [25], allowing for quick decision-making and this can therefore facilitate work for outbreak investigators. Besides fast processing, visualisation also allows for disease-specific characteristics in the assessment of the plausibility. While the first step in cluster detection, which identifies possible transmission clusters, can be done by algorithms, as it is a very generic process, the second step needs disease-specific considerations which cannot easily be incorporated in an algorithm. For example, in the case study our tools show that cluster 4 (pink) and cluster 5 (orange) span across multiple branches of the phylogenetic tree. A mumps expert knows that the mumps virus mutation rate is very low, which decreases the plausibility that these clusters represent unique transmission clusters.

An important aspect of our study is that only 5.5% of notified mumps cases had sufficient genetic information to be included. There are several reasons for this. First, mumps notification does not require laboratory confirmation in the Netherlands, but can also be based on the presence of an epidemiological link to a confirmed case. For these cases no material is available for testing and sequencing. Second, the obtained material is not always suitable for typing; viral loads can be low, which often result in failed sequencing. Third, we specifically chose to include only cases with an available sequenced sample of the SH, HN and F gene. Instead we could also have included cases with a sequenced sample of the SH gene only, as this would have resulted in a higher number of included cases. Nevertheless an earlier study [33] showed that the SH gene alone did not provide sufficient resolution for finding transmission clusters, whereas the combination of the three genes did. Since we aim to find transmission clusters here, including only sequenced samples of the SH gene was not an option. Because of these reasons, it is highly likely that the identified clusters in this study are actually larger or that some clusters are completely missed by the algorithm, as only one or two cases of a cluster might have a laboratory confirmation. This might explain the clusters reported by the NEWC (report 4, 7, 10 and 11), which were not identified by our tool (Table 1).

Our approach can handle incomplete data, such as cases with missing sequences, by performing a partial data analysis as in Supplement S2. Complete data analysis is shown in the main text. Further work can focus on extending the algorithm to allow for missing data on one or more dimensions. Especially considering that genetics information will often be missing if sequences are not available, one could replace sequence information by a categorical variable with pathogen subtype or other lower resolution indication of the pathogen type. Cases with a similar pathogen (sub)type would then have a distance of zero vs a distance of 1 to cases with another (sub)type. The (sub)type information, however, should still have sufficient resolution to be able to contribute to transmission cluster detection. Similarly, if geographical location information is not available on the latitude/longitude level, one can think of lower resolution solutions. In this study, we use the centroids of the four digit postal codes as geographical location information. The information should have sufficient resolution to be informative. The tool is not limited by the number or type of dimensions. The addition or reduction of dimensions only requires small adaptions in our code and it is therefore straightforward to use our tools in combination with other algorithms, for example, space-time algorithms [10]. By increasing or decreasing the number of dimensions in the algorithm, the relative weight of the included dimensions decreases or increases as well, respectively. Depending on the quality of the data from the additional or removed sources, this may not be desirable. We have specifically chosen not to put weights on the separate dimensions, as determining the size of the weights is a very arbitrary decision. Instead, in the current study, we would rather interpret a cluster, which was primarily identified on the geographical location dimension, as less plausible, as the geographical location data are considered quite unreliable for mumps in the Netherlands. Indeed, information on place of residence (geographical location) is of questionable accuracy as mumps mainly occur among students who often have more than one living address (near the university and their parents’ address). It is then often not clear if the students actually live on the reported address at the time of the outbreak. On the other hand, if we would have had reliable geographical location information, other or more clusters might have been identified that now go undetected. Similarly, for mumps it is very unlikely that a transmission cluster is spread across multiple branches in the phylogenetic tree, so for mumps the genetic dimension might have more relevance than e.g. the geographical one. Instead of putting a weight on this dimension however, we considered cluster 4 and 5 as less plausible. Further work could investigate ways of determining the size of the weights, based either on the quality of the data (as discussed here) or on the type and transmission routes of the disease under investigation. A final issue regarding the internal correlation plots is that they are more useful when cluster sizes are larger. If the algorithm detects very small clusters (n<4), we suggest to rely on the other tools to determine whether an identified cluster is plausible.

To conclude, our proposed tools for assessing plausibility of automatically identified clusters in time, geographical location and genetic dimensions can help outbreak investigators to focus on the most plausible clusters first. Timely availability of data are a prerequisite for this. In addition, using visual tools allow for fast and efficient information processing, which facilitates work. Mumps serves as an example in this study, but the algorithm can be transferred to other human-to-human transmissible diseases.
Conflict of interest

None declared.

Authors' contributions

LS, SH, and JW contributed to the design of the study. LS and JB led on the data analysis and drafting of the manuscript supported by SG and RvB. JW and SH supervised the whole process. All authors commented on drafts of the manuscript and approved the final version.

References


Influenza surveillance: determining the epidemic threshold for influenza by using the Moving Epidemic Method (MEM), Montenegro, 2010/11 to 2017/18 influenza seasons

Bozidarka Rakocevic1, Anita Grgurevic 1, Goran Trajkovic1, Boban Mugosa1, Sandra Sipetic Grujicic1, Sanja Medenica1, Olivera Bojovic1, Jose Eugenio Lozano Alonso6, Tomas Vega6
1. Center for Disease Control and Prevention, Institute of Public Health, Podgorica, Montenegro
2. These authors contributed equally to this work
3. Institute of Epidemiology, Faculty of Medicine, University of Belgrade, Belgrade, Serbia
4. Institute for Medical Statistics and Informatics, Faculty of Medicine, University of Belgrade, Belgrade, Serbia
5. Department for Tuberculosis, Hospital for Lung Disease and Tuberculosis Brezovik, Niksic, Montenegro
6. Public Health Directorate, Castilla y Leon Regional Health Ministry, Valladolid, Spain

Correspondence: Anita Grgurevic (anita.grgurevic@gmail.com)


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Background: In 2009, an improved influenza surveillance system was implemented and weekly reporting to the World Health Organization on influenza-like illness (ILI) began. The goals of the surveillance system are to monitor and analyse the intensity of influenza activity, to provide timely information about circulating strains and to help in establishing preventive and control measures. In addition, the system is useful for comparative analysis of influenza data from Montenegro with other countries. Aim: We aimed to evaluate the performance and usefulness of the Moving Epidemic Method (MEM), for use in the influenza surveillance system in Montenegro. Methods: Historical ILI data from 2010/11 to 2017/18 influenza seasons were modelled with MEM. Epidemic threshold for Montenegro 2017/18 season was calculated using incidence rates from 2010/11–2016/17 influenza seasons. Results: Pre-epidemic ILI threshold per 100,000 population was 19.23, while the post-epidemic threshold was 17.55. Using MEM, we identified an epidemic of 10 weeks’ duration. The sensitivity of the MEM epidemic threshold in Montenegro was 89% and the warning signal specificity was 99%. Conclusions: Our study marks the first attempt to determine the pre/post-epidemic threshold values for the epidemic period in Montenegro. The findings will allow a more detailed examination of the influenza-related epidemiological situation, timely detection of epidemic and contribute to the development of more efficient measures for disease prevention and control aimed at reducing the influenza-associated morbidity and mortality.

Introduction
Influenza is a highly infectious viral disease that represents a considerable public health problem, as it is a cause of significant morbidity and mortality rates globally [1,2]; the most severe clinical symptoms and mortality occur in high-risk populations [3]. Influenza also places an important socioeconomic burden on society, owing to the high cost of treatment and a decrease in work productivity when an individual is infected [4,5]. During the influenza season, factors such as individual awareness of the disease, social behaviour, climate, spread of influenza virus infection and discrepancies between the vaccine strain and the circulating strain of influenza virus (leading to decreased vaccine effectiveness) etc. act in concert to create an influenza epidemic [6]. The severity and duration of the influenza epidemic vary every year due to differences in virus circulation, population susceptibility and climatic factors; in addition, the onset, duration, intensity, geographic spread of influenza and the severity of the disease are often unpredictable [7-10].

The specific goal of influenza surveillance is to provide timely and high-quality epidemiological data to reduce the impact of illness and to inform public health authorities in their appropriate response to this disease. Two important benefits include the comparison of data from the current influenza season to previous seasons and the identification of an increased activity during a specific time frame, which could represent the onset of an influenza epidemic. A specific target of influenza control is the identification of epidemic thresholds that will determine the start and end
of an epidemic [11]. In order to support public health authorities in anticipating onset of influenza epidemics and initiating an appropriate and timely response, the concept of epidemic thresholds is applied in a large number of national monitoring programs [12]. There are numerous methods for calculating the onset of an influenza epidemic in the season using different data sources [9,13]. According to the World Epidemiological Standards for the Influenza Surveillance, the World Health Organization (WHO) provides several methods for determining the epidemic threshold, one of which is visual, process control and averaging [11]; Moving Epidemic Method (MEM) is an example of averaging method. For example, in Spain, a model for detection of seasonal epidemics has been applied since 2003 and a modified version of MEM has been implemented by the European Centre for Disease Control and Prevention (ECDC) in 2011/12 and the WHO in 2012/13 [14].

A comprehensive review of available literature pertaining to influenza in Montenegro has revealed an absence of studies in which one of the models for determining the threshold values for the epidemic period has been employed. The MEM method has never been used in Montenegro where surveillance of influenza-like illness (ILI) and acute respiratory infections (ARI) has been conducted since 2009 when WHO weekly reporting was also initiated.

The objective of this study was to evaluate the performance and usefulness of the MEM to establish whether it is appropriate method to be used as part of the influenza surveillance system in Montenegro.

**Method**

Montenegro is located in south-east Europe, with the population of 620,045 inhabitants. Owing to its geographical position, there are several climate zones (Mediterranean, modified Mediterranean and temperate continental climate). The Influenza surveillance system in Montenegro is designed for monitoring ILI, ARI, laboratory-confirmed influenza cases and severe
acute respiratory infection (SARI). ILI is defined as an acute respiratory illness with onset during the last 7 days with: measured temperature ≥ 38° and cough. ARI is defined as an acute onset of at least one of the following four respiratory symptoms: cough, sore throat, shortness of breath, coryza and a clinician's judgment that the illness is due to an infection. ARI may present with or without fever [15].

Population surveillance of ILI and ARI is carried out throughout the calendar year and the weekly monitoring and reporting to WHO and ECDC is carried out during the influenza season (from calendar week 40–week 20 the following year). General practitioners and paediatricians in all primary healthcare centres in the country report ILI and ARI cases through electronic registration. This information is aggregated in the central database of the Institute of Public Health Montenegro [16].

Weekly data on the number of patients by age group with ILI and ARI, as well as the number of laboratory confirmed cases of influenza during the influenza season, were obtained from the Institute of Public Health of Montenegro.
The Moving Epidemic Method (MEM)

The main purpose of the MEM is to calculate an epidemic threshold to serve as an alert signal for an upcoming epidemic. In addition, MEM calculates intensity thresholds to compare current epidemic intensity with previous epidemics identified from the same surveillance system as well as from other surveillance systems [14,17].

Using historical data (e.g. data from previous influenza seasons) from a specific surveillance system, the algorithm locates the timing of the influenza epidemic from each season (the MEM epidemic period) and separates it from pre-epidemic and post-epidemic activity. The epidemic threshold is calculated using the pre-epidemic values of historical seasons.

Intensity thresholds are calculated using the highest values of each epidemic period pooled together and calculating one-sided confidence intervals (CI) at several given levels. The three intensity threshold plus the epidemic threshold for five levels of intensity: baseline, low, medium, high and very high. In this paper,
the geometric mean and levels of 40 (medium), 90 (high) and 97.5% (very high) has been used. MEM gives estimations of the goodness of the method using a cross-validation procedure, comparing the weeks of each target season in the epidemic/non-epidemic periods (as isolated by the MEM algorithm) with weeks (of each target season) above/under the epidemic threshold calculated using the remaining seasons. In this context, sensitivity is defined as the number of epidemic weeks above the pre-epidemic threshold (before the peak) and above the post-epidemic threshold (after the peak) divided by the number of MEM epidemic weeks. Specificity pertains to the number of non-epidemic weeks below the pre-epidemic threshold (before the peak) and below the post-epidemic threshold (after the peak), divided by the number of MEM non-epidemic weeks. Positive
predictive value (PPV) is obtained by dividing the number of epidemic weeks above the threshold by the number of weeks above the threshold, while negative predictive value (NPV) is calculated as the number of non-epidemic weeks below the threshold divided by the number of weeks below the threshold [18,19].

In this study, data from 2010/11–2017/18 influenza seasons were used. For a target season all the remaining seasons were used to calculate the epidemic threshold and the three intensity thresholds (medium, high and very high); summary statistics e.g. goodness statistics, peak value of the season, the week where the peak is reached and peak intensity level were also calculated. For the 2017/18 influenza season, the number of false alerts and timeliness were also calculated. Here, false alert is defined as a weekly observed rate that is above the pre-epidemic threshold but is not in the MEM epidemic period. Timeliness pertains to the number of weeks between the alert week and the first week of the epidemic period as modelled by MEM. Statistical analysis was performed using the mem package of the R Language statistical software [20]. Graphs were produced using the memapp package, the MEM Wep Application using the Shiny Framework [21] and available from: www.memwebapp.com.

**Ethical statement**
The Ethical Committee of the Faculty of Medicine, University of Belgrade, reviewed and approved the study (No 29/III-1; 28/3/2016).

**Results**

**Modelled 2017/18 influenza season**
The historical ILI time series used in this study is shown in Figure 1, indicating that the highest activity was recorded in 2016/17 and the lowest in the 2015/16 influenza season.

The epidemic periods modelled by MEM for each season are presented in Figure 2 and 3. The pre-epidemic threshold per 100,000 inhabitants for ILI calculated for the 2017/18 target season was 19.23 while the post-epidemic threshold per 100,000 inhabitants was 17.55. In the observed 2017/18 season, by applying the MEM, an epidemic period of 10 weeks was identified. The fourth calendar week was identified as the alert week (the first week in the 2017/18 season with the disease incidence rate above the pre-epidemic threshold), as the MEM detected the epidemic onset (Figure 4). No false alerts were identified.

The timeliness for the 2017/18 season in Montenegro was 0, the alert week and the first week of epidemic period (modelled by MEM) began at the same time. The intensity of the epidemic was medium, with the peak activity above the medium threshold (40% CI) of the historical epidemic level.

The cross validation procedure showed an excellent fit of the model (Table 1). The sensitivity of the epidemic threshold for the 2017/18 season was 89%, the specificity was 99% and the positive predictive value (PPV) and negative predictive value (NPV) were 97% and 96%, respectively. Epidemic and intensity thresholds, peaks and intensity levels from the 2010/11–2017/18 influenza seasons are presented in Table 2. The epidemic thresholds were the lowest (12.70) during influenza season 2014/15, ranging from 19.23 to 20.05 in the other influenza seasons. The peak intensity level was low in seasons 2012/13 and 2015/16, very high in 2014/15 and 2016/17 and medium in all the rest.

**Discussion**
This study, is the first attempt to establish an epidemic threshold for the 2017/18 influenza season in Montenegro. To date, it has not been possible to determine the epidemic thresholds due to the lack of adequate historical data required for the analysis. The MEM was applied to data from eight influenza seasons, to calculate an epidemic threshold in Montenegro using ILI rates obtained through population surveillance for ILI during influenza seasons 2010/11–2016/17. Based on the historical data, the pre-epidemic threshold for Montenegro for the observed 2017/18 season
was ca. 19 cases per 100,000 inhabitants. Lower values of pre-epidemic thresholds (based on the number of consultations in primary healthcare clinics per 100,000 inhabitants) for the 2017/18 season were registered in Wales (10.4) and England (13.1), while slightly higher values were recorded in Scotland (34.5), Northern Ireland (26.6) and Spain (55.7) [13,22]. The epidemic period lasted for 10 weeks, which is within the range (6–25 weeks) reported in other studies [8,23-26]. According to the available evidence, the duration of the influenza season in Europe ranges 12–19 weeks [27].

In Montenegro, the intensity of the influenza epidemic during the 2017/18 season was medium, with the peak in activity recorded above the ‘high’ threshold, based on the historical epidemic values. The high values of sensitivity, specificity, PPV, NPV indicate that the model fitted the data well and was able to predict epidemic thresholds with high certainty.

As MEM is an open method, it provides flexible procedures for calculating the threshold for non-hospitalised patients. The main advantage of MEM stems from the use of the algorithm that divides the influenza season into three periods using pre-epidemic information to determine the threshold, which was successfully implemented in this study. The high sensitivity and specificity of the threshold in detecting the onset of the epidemic was also shown, despite the differences between the data collection systems used in Montenegro compared with other countries in the region and the variations in data quality. These findings confirm the effectiveness of the model in meeting the needs of public health services.

In Montenegro, 95% of population is covered by influenza surveillance (although the electronic control system does not include private healthcare institutions, very few operate in the country). Although the MEM can also be applied to historical ARI data, in this study, we used exclusively ILI data for the eight seasons included. According to the studies conducted in other countries, MEM has shown better performance when applied to the ILI data, thus justifying our research strategy [14].

In extant research in this field, the number of seasons used for calculation of epidemic threshold using MEM ranged from five to 18 [14,17]. However, authors of several extant studies used fewer than five seasons to calculate duration of the influenza epidemic [13]. Unlike other methods, MEM does not take into account viral data, it is a model based solely on simple epidemiological data and represents the most practical choice for a standard approach to be adopted in the region and in Montenegro.

Determining the onset of an influenza epidemic is very important for many reasons. Each seasonal influenza epidemic presents an organisational challenge for healthcare systems. Timely information on the onset and the intensity of the influenza epidemic, is also important for the optimal deployment of human resources especially at regional level, as well as for the provision of sufficient quantities of medications [28,29]. Such a threshold can be a reminder to vaccinate members of society that are at risk of adverse influenza effects. The epidemic threshold, among other monitoring indicators, is used to make decisions about prescription of antiviral drugs, to facilitate identification of high-risk patients and increase accuracy of clinical diagnosis, as well as prompt taking samples for laboratory testing [12].

The ECDC advocates determining the epidemic threshold by applying the MEM. The reporting in Europe started in the 2011/12 season, whereas the Euro Flu Influenza Platform (WHO European Region) has been in use since 2012/13 [17]. Consequently, following its approval, the MEM and the resulting threshold have

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**Table 2**

Epidemic and intensity thresholds, peaks and intensity levels, Montenegro, influenza seasons 2010/11–2016/17

<table>
<thead>
<tr>
<th>Influenza season</th>
<th>Peak (ILI/100,000 inhabitants)</th>
<th>Peak week</th>
<th>Epidemic threshold (ILI/100,000 inhabitants)</th>
<th>Medium threshold (ILI/100,000 inhabitants)</th>
<th>High threshold (ILI/100,000 inhabitants)</th>
<th>Very high threshold (ILI/100,000 inhabitants)</th>
<th>Peak level</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010/11</td>
<td>113</td>
<td>6</td>
<td>20.05</td>
<td>66.11</td>
<td>156.29</td>
<td>228.60</td>
<td>Medium</td>
</tr>
<tr>
<td>2011/12</td>
<td>120</td>
<td>11</td>
<td>20.05</td>
<td>69.20</td>
<td>161.36</td>
<td>234.60</td>
<td>Medium</td>
</tr>
<tr>
<td>2012/13</td>
<td>67</td>
<td>8</td>
<td>19.94</td>
<td>73.04</td>
<td>168.34</td>
<td>243.49</td>
<td>Low</td>
</tr>
<tr>
<td>2013/14</td>
<td>87</td>
<td>12</td>
<td>19.44</td>
<td>70.30</td>
<td>166.71</td>
<td>244.19</td>
<td>Medium</td>
</tr>
<tr>
<td>2014/15</td>
<td>193</td>
<td>9</td>
<td>12.70</td>
<td>63.47</td>
<td>136.07</td>
<td>190.61</td>
<td>Very high</td>
</tr>
<tr>
<td>2015/16</td>
<td>38</td>
<td>13</td>
<td>19.97</td>
<td>79.51</td>
<td>160.42</td>
<td>218.77</td>
<td>Low</td>
</tr>
<tr>
<td>2016/17</td>
<td>205</td>
<td>51</td>
<td>19.27</td>
<td>64.26</td>
<td>138.59</td>
<td>194.65</td>
<td>Very high</td>
</tr>
<tr>
<td>2017/18</td>
<td>91</td>
<td>8</td>
<td>19.23</td>
<td>69.77</td>
<td>165.91</td>
<td>243.30</td>
<td>Medium</td>
</tr>
</tbody>
</table>

ILI: influenza-like illness.
Thresholds were calculated based on data from all influenza seasons (2010/11–2017/18).
been in use in several countries for routine reporting about influenza seasons [22,23,30-32]. Epidemic thresholds are useful as a warning system, but should always be interpreted in conjunction with other available sources of information.

Our investigation was based on data pertaining to eight influenza seasons, which coincides with the period processed in the previously conducted surveys in 19/28 European Union countries [14]. Given the different conditions in Montenegro compared with those in other countries in the region, specifically differences in reporting and data collection methods as well as demographic characteristics and secular trends, the results from this study will contribute to the efforts to establish how best the model can fit available data, while also allowing the performance of various types of comparisons to be evaluated.

The establishment of one common method for analysing and interpreting the ILI data across Europe is the ultimate goal. The MEM could be a great option based on its intuitive concept, simple data requirements and flexibility compared with other sophisticated mathematical models. Influenza surveillance from Montenegro is included in the surveillance system already in place throughout Europe and therefore contributes to better quality of monitoring and surveillance of influenza epidemics in Europe.

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This article is dedicated to Professor Goran Trajkovic, who sadly passed away shortly before the article was published.

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Conflict of interest
None declared.

Authors’ contributions
BR and AG contributed to the conception and design, acquired data, interpreted data and drafted the article. AG and SSG contributed to the conception and design and provided critical revision of the intellectual content of the manuscript. BM, SM and OB contributed to the collection, analysis and interpretation of data. GT and JEL performed statistical analysis and interpreted the results. TV critically revised the importance of intellectual content. All authors provided final approval of the version to be published.

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Letter to the editor: Prevention of bacterial sexually transmitted infections (STI) in France: why not recommend using condoms and safer sex?

Eric Caumes1,2
1. Sorbonne Université, AP-HP, Hôpitaux Universitaires Pitié-Salpêtrière Charles Foix, Service de Maladies infectieuses et Tropicales, Paris, France
2. Sorbonne Université, INSERM, Institut Pierre Louis d’Épidémiologie et de Santé Publique, Paris, France

Correspondence: Eric Caumes (eric.caumes@aphp.fr)

Citation style for this article:

To the editor: I read with great interest the article by Ndeikoundam Ngangro et al. entitled ‘Bacterial sexually transmitted infections (STI) in France: recent trends and patients’ characteristics in 2016’, which showed an increased number of cases of syphilis, rectal lymphogranuloma venereum (LGV) and gonorrhoea in France between 2014 and 2016 [1]. The recommendation of the authors is ‘regular screening of patients and partners followed by prompt treatment to interrupt STI transmission’. Interestingly, the words ‘safer sex’ and ‘condom’ are not stated in the sections focussing on prevention in the article. The authors underline that ‘HIV prevention has expanded towards medical prophylaxis’ but this does not concern STI prevention, as medical prevention of HIV infection with pre-exposure prophylaxis (PrEP) does not protect against other STI and must thus be associated with condom use.

The reason why French public health specialists only recommend the test and treat approach for STI instead of prevention needs to be better explained; the continuous and marked surge of gonorrhoea and rectal LGV in men that have sex with men (MSM), for example, likely warrants more than a test and treat approach. There are at least eight reasons to worry about this continuous increase of STI: (i) re-occurrence of severe complications of gonorrhoea and syphilis with high rate of sequelae, (ii) worldwide increase of resistance to antibiotics including STI agents as recently illustrated with extensively drug-resistant (XDR) *N. gonorrhoeae* and XDR *Mycoplasma genitalium*, (iii) increase in the number of sexual partners per year among MSM, (iv) gastrointestinal (GI) and liver diseases related to the faecal-oral route of transmission particularly among MSM, (v) appearance of blood-borne diseases such as hepatitis C in the subgroup of highly sexually active MSM, (vi) discovery of possible sexual route of transmission for emerging infectious diseases (Ebola and Zika virus disease, Rift valley fever etc.), (vii) possibility for these latter diseases to be transmitted months after cure and (viii) history as story repeats itself [2].

Is it possible that sexually transmitted disease history, including AIDS history, has been largely forgotten? From a historical perspective, AIDS epidemics in MSM were preceded by an increase in the number of sexual partners, STI diagnoses (such as gonorrhoea and syphilis) and outbreaks of faecal-orally transmitted GI and liver diseases [2]. On another hand, safer sex and condom use were the main tools for tackling the AIDS epidemic before antiretroviral treatments were largely available [3].

With a continuous increase in STI, it is surprising that condom use and safer sex is not more actively promoted by public health authorities [1]. A major role of public health and infectious disease specialists should be to recall the general rules of STI prevention, i.e. condom use and safer sex—whether associated with biomedical prophylaxis or not, as the latter also carries the risk of antimicrobial resistance.

Indeed, promoting the repeated use of antibiotics for treatment of recurrent STI and the use of PrEP for prophylaxis of HIV infection is questionable, as condoms are an effective and harmless prevention tool. Moreover, this contrasts with the current recommendations for controlled use of antimicrobial therapy to lower antimicrobial resistance [4].

In conclusion, it should be highlighted that prevention of STI, through condom use and safer sex, is better, less harmful and cheaper than a cure [5]. This is particularly important with ever increasing concerns about antibiotic and antiretroviral resistance in a population where STI are increasing.
Conflict of interest

None declared.

Authors' contribution

Eric Caumes wrote the letter and approved the final version.

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This article is copyright of the authors or their affiliated institutions, 2019.
To the editor: We read with great interest Caumes’ letter to the editor and we are grateful for this opportunity to further explain the prevention of bacterial sexually transmitted infections (STI) in France. The author underlines rightfully that there is a missing comprehensive section dealing with STI prevention in our article titled ‘Bacterial sexually transmitted infections (STI) in France: recent trends and patients’ characteristics in 2016’ [1]. We agree that the conclusion might deal more explicitly with condom use and related topics (e.g. extensively drug-resistant (XDR) *Mycoplasma*, XDR *Neisseria gonorrhoeae*, oral-faecal transmitted diseases in men that have sex with men (MSM), recreational drug use, emergence of new STI). Nevertheless, this article does not recommend testing and treating instead of prevention since STI screening is one of the key strategies in STI prevention [1].

From an historical perspective, the dynamic of epidemics over the last two decades demonstrate that prevention coverage did not enable control of STI, despite the effectiveness of available prevention tools (e.g. condom use) [1]. Indeed, increases in STI are more noticeable in MSM, which may reflect increasing unprotected sex in this population [1-3]. An upsurge in STI is also observed in heterosexuals, with Chlamydia predominantly spreading in young women and men [1]. Thus, there are several STI epidemics driven by different determinants in various populations.

There is no controversy about condom effectiveness when it is consistently and correctly used, even if it might fluctuate by pathogens (e.g. human papillomavirus (HPV)) and sexual practices (e.g. foreplay) [4,5]. However, condom use is still inconsistent despite decades of sensitisation (e.g. awareness, barriers and enablers such as perceptions about condom use, partner support, self-efficacy, perceived risk for STI, condom costs, comfort in obtaining condoms, social norms); ongoing STI surveillance confirms that there is an insufficient level of condom use during penetrations in STI patients, regardless their sexual orientation. In addition, condoms are rarely used during oral sex in heterosexuals and MSM [6]. Factors resulting in less condom use therefore need to be carefully addressed through behavioural interventions and alternative prevention tools [7].

The role of asymptomatic and extra genital STI in sustaining silent epidemics should also be taken into account. Nucleic acid amplification test (NAAT) and testing recommendations have improved detection of asymptomatic and symptomatic STI and contribute to increasing number of diagnoses [1,8]. However, the volume of diagnosed and treated STI likely remains insufficient to control ongoing epidemics, even combined with present level of condom use [6].

Consequently, prevention should target the main drivers of STI epidemics to reduce STI reservoirs as well as new acquisitions. Condoms remain a corner stone in STI prevention and Santé publique France therefore actively promote their use through social marketing campaigns, free provision of condoms, sexual health education and promotion and collaborations with stakeholders (e.g. clinicians, scientists and patients’ associations).

Considering the burden of STI, invisible epidemics of undiagnosed STI, threat of multidrug resistance and the ‘reoccurrence of severe complications’ and ‘high rate of sequelae’ mentioned by Caumes, a comprehensive approach including several levels of prevention is required to control STI [8-11]. This justifies a wide range of combined interventions such as condom reimbursement by the national health insurance, systematic
Chlamydia testing in young women and men, STI clinics targeting the most exposed or vulnerable populations, test reimbursements and sexual partners notification in the framework of a national sexual health strategy [1,11-13].

Concerning HIV pre-exposure prophylaxis (PrEP) patients' follow-up primarily targets HIV transmission, but its components including sexual health counseling and STI testing can contribute to diagnosing invisible STI in highly exposed MSM. This could lead to a decrease in STI on the long-term if 'more patients are treated and transmission chains are broken' after an expected initial rise in the number of diagnoses [14,15]. We also must remember the role of STI in HIV acquisition and how valuable PrEP consultations are in preventing HIV transmission in highly exposed populations.

In conclusion, condom use remains a key component of STI prevention. Nevertheless, a comprehensive approach of STI prevention should continue, including testing and treating patients, notification of sexual partners following diagnosis, updating national guidelines and antimicrobial surveillance to prevent occurrence of multidrug-resistant pathogens, reinforce surveillance to detect emergent STI, understanding risk behaviour patterns, identifying barriers and finding means to improve access to a wide range of prevention tools.

Conflict of interest
None declared.

Authors' contributions
NNN, AV, NL and FL drafted, reviewed and approved this letter.

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