First-line tuberculosis drug resistance patterns and associated risk factors in Germany, 2008-2017

Saskia Glasauer1*, Doris Altmann2, Barbara Hauer2, Bonita Brodhun2, Walter Haas2, Nita Perumal2

1 Institute for Medical Information Processing, Biometry and Epidemiology—IBE, LMU Munich, Munich, Germany, 2 Department for Infectious Disease Epidemiology, Robert Koch Institute, Berlin, Germany

* Saskia.Glasauer@gmx.de

Abstract

Background

Drug-resistant tuberculosis (TB), especially multidrug-resistant TB (MDR-TB), poses a threat to public health. While standard surveillance focuses on Rifampicin and/or Isoniazid resistance, little is known about other resistance patterns. This study aims to identify predominant drug resistance (DR) patterns in Germany and risk factors associated with them in order to inform diagnostic and treatment strategies.

Methods

Case-based TB surveillance data notified in Germany from 2008–2017 were utilized to investigate DR and MDR-TB patterns for Isoniazid (H), Rifampicin (R), Pyrazinamide (Z), Ethambutol (E), and Streptomycin (S). Predominant patterns were further analyzed stratified by sex, age, country of birth, prior TB, and disease site. Multivariable logistic regression was conducted to determine risk factors associated with any resistance, MDR-TB, and complete HRZES resistance.

Results

26,228 cases with complete DST results were included in the study, among which 3,324 cases had any DR (12.7%). Four patterns were predominant, representing about ¾ of all cases with any resistance (S: 814 [3.1%]; H: 768 [2.9%]; HS: 552 [2.1%]; Z: 412 [1.6%]). High proportions of S and H resistances were found among both German and foreign-born populations, especially those born in Eastern Europe, and were unexpectedly high among children (H: 4.3%; S: 4.6%). Foreign-born cases had significantly higher proportion of any resistance (16.0%) and MDR-TB (3.3%) compared to German-born cases (8.3% and 0.6%). Of 556 MDR-TB cases, 39.2% showed complete HRZES resistance. Logistic regression revealed having prior TB and being foreign-born as consistently strong risk factors for any DR, MDR-TB, and complete HRZES resistance.
Conclusions

DR patterns observed in Germany, particularly for MDR-TB were more complex than expected, highlighting the fact that detailed drug-testing results are crucial before incorporating HRZES drugs in MDR-TB treatment. Furthermore, the relatively high rate of H-resistance in Germany provides strong rationale against the use of only H-based preventive therapy for LTBI.

Introduction

Tuberculosis (TB) is the ninth leading cause of death worldwide and the leading cause of death from a single infectious agent, exceeding even HIV/AIDS [1]. The World Health Organization (WHO) estimates that in 2017, approximately 10 million people were diagnosed with TB and 1.6 million people died due to the disease [1]. About one quarter of the world’s population is further estimated to be latently infected [1]. As a result, TB control is a top priority on the global health agenda. One of the WHO’s End TB strategy targets aims to reduce the global incidence to <100 TB cases per million by 2035 [2]. The WHO has also developed a Framework for low-incidence countries [3], asking them to reach pre-elimination phase (<10 cases per million) by 2035 and elimination (<1 case per million) by 2050 or earlier [3]. One of the biggest obstacles in achieving TB elimination is the emergence of drug resistance [1]. In 2017, 558,000 cases were estimated to be resistant to Rifampicin (R), the most effective first-line anti-tuberculosis drug [1], with 82% suffering from multidrug-resistant TB (MDR-TB) [1], which is defined as the resistance to at least Rifampicin and Isoniazid, another potent anti-TB drug [1]. Tackling MDR-TB is an especially important cornerstone of the increasingly urgent global fight against antimicrobial resistance (AMR) [4].

A number of experts have suggested the lack of political commitment, inadequate healthcare systems, poor disease management, unsound drug policies, and a long-standing neglect in research as having facilitated the global rise in drug-resistant TB and MDR-TB [5, 6]; the increase in MDR-TB is especially concerning due to its prolonged, difficult, and potentially toxic treatment. In 2016, in the European Economic Region, almost two-thirds of MDR-TB cases were notified as having failed, defaulted, or died during treatment [7]. Inadequate MDR-TB treatment facilitates the development of extensively drug resistant TB (MDR-TB plus resistance against one Fluoroquinolone and one injectable; XDR TB) and paves the way for further transmission [5]. Thus, resistant, and especially MDR-TB, pose an immense threat to public health [8].

With almost 20% of R-resistant (RR) or MDR-TB cases in 2016, the WHO European Region has the highest regional burden of MDR-TB in the world [6, 9]. In 2016 alone, 19% of new TB cases and 55% of previously treated cases were diagnosed with RR/MDR-TB, which is considerably higher than the global average [10]. Eastern European countries present the highest resistance rates in Europe [6, 11]. Studies have further shown that MDR-TB strains have been imported into and transmitted throughout the EU/EEA region [5, 11–14].

Germany represents a low TB incidence country, with 5,486 cases in 2017 and a notified incidence of 6.7/100,000 [15]. In the past 10 years, the drug resistance trend in Germany has fluctuated slightly, peaking in 2013, but has remained overall stable at 2–3%. In 2017, drug resistance against at least one of the five standard anti-TB drugs H, R, Pyrazinamide (Z), Ethambutol (E) and Streptomycin (S) was notified in 431 (11.9%) cases with available information (information needed be available for at least H and R resistances). MDR-TB was reported in...
109 cases (3.0%), of which 4 were reported as having XDR-TB [15]. Even within the context of Germany's low TB incidence, however, it is important to remain attentive to epidemiological changes in drug-resistant TB in order to inform clinical decision-making on appropriate drug regimens [16].

As TB treatment has shifted from standardized to more individualized approaches [17–19] information on drug resistance patterns within the population has become ever more relevant and important. Furthermore, due to the high prevalence of MDR-TB among R-resistant cases, R resistance is considered as surrogate for MDR-TB and amongst the WHO-endorsed rapid drug susceptibility testing (DST) tools, one of the most frequently utilized tools tests only for R resistance [20–23]. However, this approach is questioned by some experts and, hence, additional DST for H, especially in low-incidence settings, is recommended [24, 25]. Here too, information on drug resistance patterns is crucial to inform the utilization of different resistance diagnostic methods.

To our knowledge, with the exception of one study conducted by the European Centre for Disease Prevention and Control, covering a period from 2007 to 2012, there is currently no analysis available on drug resistance patterns in low TB incidence countries in Europe, such as Germany [16]. Other studies who have addressed this question either limited their analysis to certain population groups [26–28] or were conducted using surveillance data from high TB incidence countries with very different TB epidemiology [29–31]. As a result, this study aims to analyze German national TB surveillance data for the predominant drug resistance patterns for the five standard anti-TB drugs (Isoniazid—H, Rifampicin—R, Pyrazinamide—Z, Ethambutol—E, Streptomycin—S; HRZES) and the factors associated with them, in order to inform diagnostic and treatment strategies for TB patients in low incidence countries such as Germany. The objectives of this study are to determine (i) frequency and distribution of drug resistance patterns over a 10-year period and (ii) demographic factors associated with specific drug resistance patterns.

**Methods**

**Data source and collection**

TB is a notifiable disease in Germany [32]. District health authorities report, via their respective state health departments case-based data electronically to the national surveillance database (SurvNet@rki, [33]) of the Robert Koch Institute (RKI). The data comprises of, among others, information on case demographics, site of disease, prior TB disease, bacteriological findings, DST results, and treatment outcome.

**Case inclusion and definitions**

For study inclusion, a case had to meet the following criteria: fulfill the RKI reference case definition for active TB disease [34], be notified between the years 2008 to 2017, be culture positive, and have been tested for all five HRZES drugs and have DST results available. The cut-off date for the data extraction was March 1st, 2018. As S was considered and used as a first-line TB drug until 2011 covered by the study period, it was included in the analyses to reflect drug resistance dynamics in Germany. Z was also included, despite inconsistent international guidelines regarding the reliability and quality assurance of its DST [35, 36] as interlaboratory tests in Germany revealed good performance; Z has become increasingly important in newer therapy regimens [37, 38]. Information on second-line drugs could not be included in this analysis due to insufficient data completeness.

Cases < 15 years were categorized as children; cases between 15 and 59 years were categorized as adults, and cases ≥ 60 years as elderly. Any drug resistance was defined as resistance...
against at least one of the HRZES drugs, any R and any H resistance was defined as resistance to at least R or H, respectively. MDR-TB was defined as being resistant against at least H and R. A case was considered a previous case, when a prior diagnosis of TB disease was notified.

Data analysis

For the purposes of this study, the following variables were extracted from the national surveillance database: age, sex, country of birth, prior TB diagnosis (yes/no; used as surrogate for prior treatment due to a much higher level of data completeness), main site of disease (pulmonary vs. extrapulmonary), presence of MDR-TB (yes/no), individual DST results for HRZES (resistant vs. susceptible). For children <15 years of age, country of birth (born in Germany vs. born outside Germany) of the father and/or mother were also extracted.

Drug resistance patterns and their distribution were first analyzed descriptively and presented as case counts and proportions stratified by sex, age-group, country of birth, prior TB, and site of disease. Trends in HRZES monoresistances and MDR-TB, including MDR-TB with additional resistances to first line drugs, were analyzed using the Chi² test for linear trends. For all other analyses, data were pooled over the entire study period. Case counts were compared using the Chi² test and the corresponding p-values specified.

Further exploratory analysis was then conducted using univariable logistic regression. Finally, multivariable logistic regression analyses were performed to determine the association between age, sex, country of birth, previous TB, and site of disease (independent variables) and the presence of any resistance, MDR-TB, and resistance against all five drugs (dependent variables). The variable age was categorized into the following three groups before inclusion into the models: children (<15 years), adults (15–59 years) and elderly (60+ years). This was conducted in order to elucidate the expected differences in drug resistance patterns between the different age groups due to birth cohort effects as well as effect modification by age. Cases with missing values for any of the included variables were excluded from the analyses. A p-value of <0.01 was considered significant for the descriptive analyses, while 95% confidence intervals were calculated for the logistic regression analyses. All data were analyzed in RStudio Version 1.0.153 [39]. All data were collected in accordance with the German ‘Protection against Infection Act’ (‘Infektionsschutzgesetz’) and data protection guidelines were strictly followed. Informed consent was deemed not necessary as data were fully anonymized before analysis.

Results

Between 2008 and 2017, 48,044 active TB cases that met the RKI reference definition were notified in Germany. 41,932 cases (87.3%) had a culture result available, of which 34,141 were culture positive (81.4%). Of these, DST results were available for all 5 drugs (HRZES) for 26,228 cases or 76.8% (Fig 1). These 26,228 cases were considered to be the final study population.

For those with available information, foreign-born cases comprised 57.1% of all included cases. Similarly, for those with available information, the median age of the entire study population was 45 years and 63.2% were male. Median age among foreign-born cases was 35 years and 62.3% were male (German-born: 58 years and 64.7%, respectively). 12.5% of all cases with information on prior diagnosis were previously diagnosed with TB. Further case characteristics are shown in Table 1.

Trends in drug resistance over time

The pooled national data revealed an overall proportion of any drug resistance of 3,324/26,228 (12.7%) in Germany (Fig 1). No significant overall changes could be observed for H, R, Z, and
E monoresistance rates between 2008 and 2017 (p > 0.01 for all; Fig 2), while S-monoresistance rates showed a significantly increasing trend over time (p < 0.01, respectively) (Fig 2). However, the increasing trend was present until 2016 and the S-monoresistance rates declined in 2017. Overall, MDR-TB was found in 2.1% of cases in Germany but showed a significantly increasing trend over the study period (p < 0.01; Figs 2 and 3). MDR-TB accounted for 40/2,891 (1.4%) TB cases in 2008 and for 47/1830 (2.6%) in 2017. Among MDR-TB cases, no clear trend was observed in the rates of resistance to all five drugs, but a remarkable increase from 2012 (10/54 (18.5%) of MDR-TB cases) to 2013 (46/87, 52.9% of MDR-TB cases) (Fig 3).

Resistance patterns overall
Among the 26,228 cases with complete DST results, 3,324 (12.7%) were resistant to at least one of the five drugs (any resistance) (Fig 1). Out of 31 possible resistance patterns, 28 could be identified in the surveillance data, of which 4 patterns accounted for more than ¾ of all drug resistant cases (for S: 24.5%; H: 23.1%; HS: 16.6%; Z: 12.4%; Fig 1). Three possible patterns were not represented (RZE, RZS and ZES) and one pattern was only found once (HZE). Overall, 556 (2.1%) cases had MDR-TB, of which 218/556 (39.2%) showed resistance against all five drugs, 143/556 (25.7%) were resistant to four drugs, 141/556 (25.4%) to three drugs and 54/556 (9.7%) were resistant to only H and R (Fig 1).
Any resistance to R was present in 643/3,324 (19.3%) cases and 556 (86.5%) of them had MDR-TB, i.e. additional resistance to at least H. 87 R resistant cases had no accompanying H resistance (Table 2), accounting for 0.3% of all included cases and 13.5% of all cases with any R resistance.

### Drug resistance according to sex

Within the study population, 9,627/26,228 (36.8%) cases were female and 16,556/26,228 (63.2%) were male (Table 1). 2,033/16,556 (12.3%) males and 1,280/9,627 (13.3%) females diagnosed with TB had any resistance. No significant difference between sexes could be observed with regard to any resistance, H mono-resistance, combined resistance to HS and MDR-TB (p > 0.01 for all, data not shown). However, females had a significantly higher proportion of Z- mono-resistance (male: 222/2,033, 10.9%; female: 190/1,280, 14.8%, p < 0.01), while males had a significantly higher proportion of S- mono-resistance (male: 534/2,033, 26.7%; female: 279/1,280, 21.8%, p < 0.01).

### Drug resistance according to country of birth

When examining the resistance patterns by country of birth, cases born in Russia, Somalia, Kazakhstan, Turkey, India, Vietnam, Romania, Eritrea, Pakistan and Afghanistan accounted...
for the highest numbers of drug resistant cases. Cases born in Russia, Kazakhstan and Vietnam presented the highest proportions of any drug resistance with 33.2%, 28.2%, and 26.7%, respectively. For MDR-TB, once again cases born in Russia (14.1%) and Kazakhstan (8.2%) presented the highest proportions, followed by Somalia (3.8%). Because of the recent shift in country of origin of migrants in Germany from predominantly Eastern European countries towards countries from the Horn of Africa and the Middle East, country rankings were also analyzed in two 5-year timespans (2008–2012 and 2013–2017). Between 2008 and 2012, Turkey, Russia, and Kazakhstan, had the three highest absolute shares of resistances in Germany. However, between 2013 and 2017, African countries became more dominant and cases born in Somalia and Eritrea, having the top two absolute shares of resistances, with Romania ranked third. Table 3 shows the overall proportions of any drug resistance, any H-resistance, any R-resistance, and MDR-TB for the top 10 countries of birth from 2008 to 2017.

Resistance patterns according to age and country of birth

Overall, German-born cases had a significantly lower proportion of any resistance than foreign-born patients (German-born: 913/10,969, 8.3%, Foreign-born: 2,337/14,600 16.0%, p<0.01). In German-born cases, drug resistance was mainly attributable to monoresistances, especially H (228/913, 25.0%) and S (236/913, 25.8%; Table 4). Moreover, 96 German-born cases had any resistance to R (0.9%), among which 70 (72.9%) had MDR-TB and 20 (20.8%) had R monoresistance. In foreign-born cases, drug resistance was attributable to high proportions of MDR-TB (478/2,337, 20.5%), H (521/2,337, 22.3%) and S (561/2,337, 24.0%) monoresistances. 536 (3.7%) foreign-born cases had any R-resistance of which 478 (89.2%) cases had...
MDR-TB and 48 (9.0%) cases had R monoresistance. Foreign-born cases also had higher proportions of combined resistance against HS (foreign-born: 429/2,337, 18.4%, German-born: 109/913, 11.9%; p<0.01), especially in the age groups 40–59 years (Foreign-born: 150/633, 23.7%; German-born: 45/287, 15.7%; p<0.01) and 60+ years, with a notable difference in those aged 60+ (German-born: 28/395, 4.6%; foreign-born: 56/283, 19.8%; p<0.01). However, cases born in Germany had a higher proportion of Z-monoresistance (German-born: 206/913, 22.6%; foreign-born:197/2,337 8.4%, p<0.01), especially among those aged 60+ (German-born: 145/395, 36.7%; Foreign-born: 44/283, 15.5%, p<0.01).

Both groups showed a decreasing MDR-TB trend with increasing age (p<0.01 for both). However, cases born outside Germany were more affected by MDR-TB with 478/14,600 (3.3%) compared to 70/10,969 (0.6%) in German-born cases (p<0.01). For cases with MDR-TB, regardless of their origin, resistance against all 5 drugs was the most common pattern, accounting for 23/70 (32.9%) of MDR-TB cases born in Germany and 191/478 (40.0%) of cases born outside Germany (Table 5). Resistance against only H and R accounted for 10/70 (14.3%) of all MDR-TB cases among German-born cases and for 44/478 (9.2%) of cases born outside Germany.

**Drug resistance according to prior TB**

Overall, 2,820/22,555 (12.5%) of all cases reported having prior TB diagnosis, while the majority (19,735/22,555, 87.5%) reported not having a prior disease. Cases with prior TB had a significantly higher proportion of any resistance, with 516/2,820 (18.3%) in comparison to 2,303/19,735 (11.7%) among new cases (p<0.01). While new cases presented significantly higher
proportions of S (new cases: 614/2,303, 26.7%; previous cases: 81/516, 15.7%; p < 0.01) and Z monoresistance (new cases: 300/2,303, 13.0%; previous cases: 43/516, 8.3%; p < 0.01), previous cases had a higher MDR-TB proportion with 186/2,820 (6.6%) in comparison to 281/19,735 (1.4%, p < 0.01) for new cases. Among MDR-TB cases, resistance against all five drugs (HRZES) was once again the predominant pattern for both groups, accounting for 92/281 (32.7%) of new and 89/186 (47.8%) of previous MDR-TB cases. After further stratification for country of birth, only foreign-born cases still showed significant differences in the proportions of any resistance and MDR-TB between new and previous cases. Specifically, 1,553/10,630 (14.6%) of foreign-born new cases and 367/1,352 (27.1%) of previous cases had any resistance and 224/10,630 (2.1%) of foreign-born new cases and 171/1,352 (12.6%) of previous cases had MDR-TB.

Drug resistance according to site of disease
Within the study population 21,254/26,195 cases (81.1%) had pulmonary TB and 4,941/26,195 (18.9%) cases had extrapulmonary TB. For any resistance, there was no significant difference

| H | R | Z | E | S | Number of cases | Percentage of cases |
|---|---|---|---|---|------------------|---------------------|
|   | x |    |   | x | 816              | 24.6%               |
| x |    |    |   | x | 769              | 23.1%               |
| x |    |    |   | x | 551              | 16.6%               |
|   | x |    |   |   | 412              | 12.4%               |
| x | x | x | x | x | 218              | 6.6%                |
| x | x |    | x | x | 112              | 3.4%                |
| x |    | x |    |   | 70               | 2.1%                |
| x | x | x | x |   | 67               | 2.0%                |
| x | x |    | x | x | 65               | 2.0%                |
| x | x |   | x |   | 54               | 1.6%                |
|   | x | x |   |   | 36               | 1.1%                |
| x |    |   | x |   | 27               | 0.8%                |
| x | x |    |   |   | 24               | 0.7%                |
| x | x | x |   |   | 19               | 0.6%                |
|   | x |   | x |   | 13               | 0.4%                |
| x | x | x | x |   | 12               | 0.4%                |
| x | x | x |   |   | 11               | 0.3%                |
| x | x |   | x |   | 10               | 0.3%                |
| x |   | x |   |   | 10               | 0.3%                |
| x | x |    |   |   | 5                | 0.2%                |
| x |    |   | x |   | 5                | 0.2%                |
| x | x |   | x |   | 5                | 0.2%                |
| x | x |   |   |   | 3                | 0.1%                |
| x | x | x | x |   | 2                | 0.1%                |
| x | x | x | x |   | 2                | 0.1%                |
| x |   | x | x |   | 1                | 0.0%                |
| x |   | x |   | x | 0                | 0.0%                |
| x | x |   | x |   | 0                | 0.0%                |

https://doi.org/10.1371/journal.pone.0217597.t002
between pulmonary TB cases (2,646/21,254, 13.6%) and extrapulmonary TB cases (671/4,941, 12.4%, p < 0.01). For monoresistances, however, cases with pulmonary TB had a significantly lower proportion of Z monoresistance (pulmonary: 263/2,646, 9.9%; extrapulmonary: 148/671, 22.1%, p < 0.01). In contrast, cases with pulmonary TB had a higher occurrence of MDR-TB with 493/21,254 (2.3%) in comparison to 62/4,941 (1.3%) of cases with extrapulmonary TB (p < 0.01). Full resistance against HRZES was once again predominant among MDR-TB cases, being reported in 196/496 (39.8%) of pulmonary MDR-TB cases and 22/62 (35.5%) of extrapulmonary MDR-TB cases.

Regression analyses

The final multivariable logistic regression results are presented in Table 6. Compared to adults, children had higher odds of having any drug resistance (OR = 1.59; 95% CI: 1.27, 1.97), MDR-TB (OR = 1.74; 95% CI: 1.03, 2.77), and resistance against all five drugs (OR = 2.08; 95% CI: 1.24, 4.42). In contrast, elderly had lower odds for all three resistance patterns compared to adults. Males had significantly lower odds of having any resistance in comparison to female cases (OR = 0.90; 95% CI: 0.84,0.98), but no significant effect of sex could be found for MDR-TB and resistance against all five drugs. Cases born outside of Germany had almost twice the odds of having any resistance versus cases born in Germany (OR = 2.04; 95% CI: 1.87,2.22). This increased to almost 4.5 times higher odds for having MDR-TB (OR = 4.4.71; 95% CI: 3.70,6.10) and a more than 3 times higher odds for having resistance against all five drugs (OR = 3.86; 95% CI: 2.51,6.22). Cases with prior TB diagnosis had approximately double the odds of having any resistance versus those with no prior TB diagnosis (OR = 1.95; 95% CI: 1.76,2.16); this increased to almost 6 times higher odds for MDR-TB (OR = 5.95; 95% CI: 5.00,7.06) and 9 times higher odds for resistance against all five drugs (OR = 9.01; 95% CI: 6.66,12.19). Lastly, cases with pulmonary TB had twice the odds of having both MDR-TB (OR = 2.00; 95% CI = 1.57,2.61) and resistance against all five drugs (OR = 2.12; 95% CI: 1.33,3.60) versus cases with extrapulmonary TB.

Discussion

Our study is one of the limited number of recent studies worldwide to analyze national surveillance data on drug resistance patterns over a long period of time. Our analysis revealed an
overall any drug resistance proportion of 12.7% in Germany, among which four resistance patterns were clearly predominant and accounted for more than \( \frac{3}{4} \) of all drug resistant cases in monoresistance against S, monoresistance against H, combined resistance against HS and monoresistance against Z. Monoresistance against S was the most prevalent drug resistance pattern in cases born in Germany and outside Germany, as well as among all age groups, very likely due to the long historical usage. These findings confirm that the withdrawal of S as a first-line drug in 2012 in Germany [38] has minimal relevance for TB therapy due to high resistance levels against it in the population. As a result, it can be questioned whether systematic surveillance of S resistance is still essential.

Resistance against H presents another highly prominent drug resistance pattern among tuberculosis patients in Germany. Our analysis showed high proportions of H resistance among all subpopulations, but cases originating from Eastern Europe showed remarkably high proportions of resistance. This supports the use of either 3–4 months of combined H and R therapy or 4 months of R monotherapy for LTBI treatment in Germany [19].

### Table 4. The 4 predominant patterns in drug-resistant culture positive TB cases with DST results, by country of birth and age group (for cases with available information), Germany 2008–2017, Isoniazid (H), Rifampicin (R), Pyrazinamide (Z), Ethambutol (E), and Streptomycin (S).

| Age group: < 15 yr. | Country of birth | Total No. of TB cases | Any resistance present | Among any resistance |
|---------------------|-------------------|-----------------------|-----------------------|---------------------|
|                     | Germany           | 366                   | 64 (17.5%)            | H 16 (25.0%)        |
|                     | Other             | 209                   | 39 (18.7%)            | Z 6 (9.4%)          |
|                     |                   | 575                   | 103 (17.9%)           | S 20 (31.3%)        |
|                     |                   |                       |                       | HS 12 (18.8%)       |
| Age group: 15–39 yr. | Germany           | 1,979                 | 167 (8.4%)            | H 50 (29.9%)        |
|                     | Other             | 8,323                 | 1,381 (16.6%)         | Z 10 (6.0%)         |
|                     |                   | 10,302                | 1,548 (15.0%)         | S 302 (21.9%)       |
|                     |                   |                       |                       | HS 111 (7.2%)       |

| Age group: 40–59 yr. | Country of birth | Total No. of TB cases | Any resistance present | Among any resistance |
|---------------------|-------------------|-----------------------|-----------------------|---------------------|
|                     | Germany           | 3,449                 | 287 (8.3%)            | H 71 (24.7%)        |
|                     | Other             | 3,542                 | 633 (17.9%)           | Z 46 (7.3%)         |
|                     |                   | 6,991                 | 920 (13.2%)           | S 132 (20.9%)       |
|                     |                   |                       |                       | HS 119 (18.8%)      |

| Age group: 60+ yr. | Country of birth | Total No. of TB cases | Any resistance present | Among any resistance |
|-------------------|------------------|-----------------------|-----------------------|---------------------|
|                    | Germany          | 5,174                 | 395 (7.6%)            | H 91 (23.0%)        |
|                    | Other            | 2,525                 | 283 (11.2%)           | Z 78 (27.6%)        |
|                    |                   | 7,699                 | 678 (8.8%)            | S 44 (15.5%)        |
|                    |                   |                       |                       | HS 169 (24.9%)      |

| All age groups     | Country of birth | Total No. of TB cases | Any resistance present | Among any resistance |
|--------------------|------------------|-----------------------|-----------------------|---------------------|
|                    | Germany          | 10,969                | 913 (8.3%)            | H 228 (24.9%)       |
|                    | Other            | 14,600                | 2,336 (16.0%)         | Z 206 (22.6%)       |
|                    |                   | 25,569                | 3,249 (12.7%)         | S 236 (25.8%)       |
|                    |                   |                       |                       | HS 99 (10.8%)       |

https://doi.org/10.1371/journal.pone.0217597.t004
We also found unexpectedly high proportions of S and H resistances among children. A potential explanation for this finding might be the presence of selection bias as bacteriological confirmation is challenging in paucibacillary childhood TB and diagnosis may, as a result, be intensified particularly in children with unknown infection sources and suspected drug resistance.

Z monoresistance was another significant finding in our analysis. We saw a remarkably high proportion of Z resistance among the German-born elderly population (60+), being almost 2.5-times higher than the proportion in the foreign-born elderly. One hypothesis might be that elderly cases present higher proportions of prior, agriculturally-acquired infection with *Mycobacterium bovis*, which is inherently resistant to Z. In fact, of the 145 German-born elderly cases with monoresistance to Z, 95 (65.5%) were reported as having *M. bovis* as the species of infection. Apart from that, Z-monoresistance was 2.2-times higher in extrapulmonary cases in comparison to pulmonary cases. Some studies have previously indicated that infection with *M. bovis* more often leads to extrapulmonary TB disease [40, 41]. As a result, species

---

### Table 5. Multidrug resistance patterns in drug-resistant culture positive TB cases with DST results, by country of birth and age group (for cases with available information), Germany 2008–2017, Isoniazid/H, Rifampicin/R, Pyrazinamide/Z, Ethambutol/E, and Streptomycin/S.

#### Age group: ≤ 15 yr.

| Country of birth | Total No. of TB cases | MDR-TB present | HR only | 3 drugs | 4 drugs | All 5 drugs |
|------------------|-----------------------|----------------|---------|---------|---------|-------------|
| Germany          | 366                   | N              | %       | N       | N       | N           |
| Other            | 209                   | 8              | 3.8%    | 1       | 1       | 2           |
| Total            | 575                   | 14             | 2.4%    | 1       | 3       | 4           |

#### Age group: 15–39 yr.

| Total No. of TB cases | MDR-TB present | HR only | 3 drugs | 4 drugs | All 5 drugs |
|-----------------------|----------------|---------|---------|---------|-------------|
| Germany               | 1,979          | 19      | 1.0%    | 2       | 3           |
| Other                 | 8,323          | 318     | 3.8%    | 27      | 79          |
| Total                 | 10,302         | 337     | 3.3%    | 29      | 82          |

#### Age group: 40–59 yr.

| Total No. of TB cases | MDR-TB present | HR only | 3 drugs | 4 drugs | All 5 drugs |
|-----------------------|----------------|---------|---------|---------|-------------|
| Germany               | 3,449          | 29      | 0.8%    | 5       | 7           |
| Other                 | 3,542          | 122     | 3.4%    | 11      | 33          |
| Total                 | 6,991          | 151     | 2.2%    | 16      | 46          |

#### Age group: 60+ yr.

| Total No. of TB cases | MDR-TB present | HR only | 3 drugs | 4 drugs | All 5 drugs |
|-----------------------|----------------|---------|---------|---------|-------------|
| Germany               | 5,174          | 16      | 0.3%    | 3       | 6           |
| Other                 | 2,525          | 30      | 1.2%    | 5       | 9           |
| Total                 | 7,699          | 46      | 0.6%    | 8       | 15          |

#### All age groups

| Total No. of TB cases | MDR-TB present | HR only | 3 drugs | 4 drugs | All 5 drugs |
|-----------------------|----------------|---------|---------|---------|-------------|
| Germany               | 10,969         | 70      | 0.6%    | 10      | 18          |
| Other                 | 14,600         | 478     | 3.3%    | 44      | 123         |
| Total                 | 25,569         | 548     | 2.1%    | 54      | 139         |

https://doi.org/10.1371/journal.pone.0217597.t005
specification of TB infection is an important step to support appropriate design of treatment regimens.

Between 2008 and 2017, 2.1% of all cases in Germany had MDR-TB. In comparison to the European average of 3.7% in 2016, Germany presents a low proportion of MDR-TB cases [7]. Nevertheless, in contrast to the European-wide trend [7], we observed a slight increase in MDR-TB cases notified over the last 10 years, the reasons for which are likely the increase in migration from high MDR-burden regions, but perhaps also better reporting by local health authorities. Especially of note is that we rarely observed “simple” MDR-TB, i.e. resistance to only H and R and MDR-TB cases mostly presented additional drug resistances. Approximately 40% of MDR-TB cases in our study were resistant to all 5 drugs (HRZES) and only 10% were resistant to H and R only. This supports the WHO recommendation to initiate MDR-TB treatment with four second-line anti-TB drugs and not to rely on any first-line drugs, until detailed DST results are available [18].

Cases with prior TB disease and foreign-born cases have a high risk of MDR-TB. Our analysis showed that cases with prior TB diagnosis had an almost six times higher odds of having MDR-TB and nine times higher odds of having resistance against all five drugs in comparison to new cases. A number of studies have analyzed risk factors associated with MDR-TB [42–53] for different populations around the world. All of them found prior treatment to be one of the most significant and consistent predictors for the presence of drug resistance. In our study, although we utilized prior TB diagnosis as a proxy for prior TB treatment, our results are consistent with these previous results. Nevertheless, MDR-TB, and especially resistance against all five drugs, was also prevalent in new cases and in children, confirming that MDR-TB strains are endemic in many settings and that MDR-TB is no longer only a matter of acquired drug resistance.

Cases born outside Germany had a 5.5-fold higher proportion of MDR-TB in comparison to cases born in Germany. Several studies have also found higher proportions of MDR-TB among migrants in comparison to the local populations [44, 47, 48, 50, 51].

| Risk factor         | Any drug resistance | Multidrug resistance (MDR-TB) | Resistance against all five drugs (HRZES) |
|---------------------|---------------------|-----------------------------|------------------------------------------|
| OR                  | 95% CI              | OR                          | 95% CI                                  | OR                          | 95% CI                                  |
| Age (years)         |                     |                             |                                         |                             |                                         |
| Adult (<15)         | ref                 | ref                         | ref                                     | ref                         | ref                                     |
| Child (15–59)       | 1.59                | 1.27                        | 1.97                                    | 1.74                        | 1.03                                    | 2.77                                    | 2.02                        | 0.78                        | 4.30                                    |
| Elderly (60+)       | 0.64                | 0.58                        | 0.70                                    | 0.24                        | 0.18                                    | 0.32                                    | 0.12                        | 0.05                        | 0.23                                    |
| Sex                 |                     |                             |                                         |                             |                                         |
| Female              | ref                 | ref                         | ref                                     | ref                         | ref                                     |
| Male                | 0.90                | 0.84                        | 0.98                                    | 0.95                        | 0.80                                    | 1.13                                    | 0.81                        | 0.60                        | 1.11                                    |
| Country of birth    |                     |                             |                                         |                             |                                         |
| Germany             | ref                 | ref                         | ref                                     | ref                         | ref                                     |
| Not Germany         | 2.04                | 1.87                        | 2.22                                    | 4.71                        | 3.70                                    | 6.10                                    | 3.85                        | 2.50                        | 6.20                                    |
| Prior TB            |                     |                             |                                         |                             |                                         |
| No                  | ref                 | ref                         | ref                                     | ref                         | ref                                     |
| Yes                 | 1.95                | 1.76                        | 2.16                                    | 5.95                        | 5.00                                    | 7.06                                    | 9.06                        | 6.66                        | 12.26                                   |
| Main site of TB     |                     |                             |                                         |                             |                                         |
| Extrapulmonary      | ref                 | ref                         | ref                                     | ref                         | ref                                     |
| Pulmonary           | 0.98                | 0.89                        | 1.07                                    | 2.00                        | 1.57                                    | 2.61                                    | 2.17                        | 1.36                        | 3.69                                    |

Table 6. Results of multivariable logistic regression analysis of factors associated with any drug resistance (HRESZ), multidrug resistant TB, and resistance against all five drugs (HRZES), 2008–2017.

https://doi.org/10.1371/journal.pone.0217597.t006
Europe, especially Russia and Kazakhstan, accounted for a substantial proportion of cases with MDR-TB in our study. This is in line with findings from Falzon and colleagues [50] showing that MDR-TB is strongly associated with origin from the former Soviet countries. Surveillance figures further show that Eastern European countries account for 85% of the TB burden and 99% of the MDR-TB cases in the WHO European region [9, 50]. However, due to the arrival of a substantial number of refugees and asylum seekers into Germany from 2014–2016, the migration pattern in Germany has undergone a change. For example, African countries, among them countries with a high burden of drug resistant TB, such as Somalia and Eritrea, have recently become prominent in the drug resistance landscape, accounting for a considerable proportion of drug resistant cases. Between 2013 and 2017, cases from Somalia and Eritrea accounted for 11.2% and 7.2%, respectively, of all foreign-born cases with any resistance (2008–2013: 2.3% and 0.7%) and for 10.9% and 2.9% of all foreign-born MDR-TB cases (2008–2013: 1.0% for both). MDR-TB cases from countries in the Horn of Africa, such as Somalia and Eritrea, were also the subject of a large Europe-wide outbreak investigation in 2016, where the majority of the cases in the cluster were reported in Germany [54]. Nevertheless, the overall proportion of MDR TB remained stable at 2–3%, and we also found MDR-TB in cases born in Germany, especially among younger, German-born cases, which highlights the continued need for rapid DST and strengthened bacteriological confirmation among all age groups.

Rapid DST techniques are meaningful, especially in patients with high risk of drug-resistant TB, in order to rapidly commence adequate treatment before phenotypic DST results become available. Recently, R resistance has increasingly been considered as a surrogate marker for MDR-TB [23]. Although R mono-resistance is rare worldwide [38], it seems to be prevalent in Germany. 13.5% of R-resistant cases in our study did not have H-resistance and among those without accompanying H-resistance, about 80% of all R-resistant cases were mono-resistant.

Together with the high proportion of H resistance in Germany and our finding that MDR-TB is rarely only HR resistance in Germany, our results raise some concerns about the possibility of incorrect diagnosis of drug-resistant TB in Germany if currently available rapid tests were to be used as the only diagnostic tests. Focusing on only R resistance or HR resistance, other important and relevant resistance patterns would be overlooked unless additional testing is conducted [55]. Although more resource-intensive, comprehensive genotypic testing using whole genome sequencing has shown high concordance with conventional phenotypic techniques for first line TB drugs and could, therefore, be another relevant rapid test technology in resource-rich countries in the future [55].

In our multivariable regression analyses, we found that being born outside of Germany and prior TB disease diagnosis were the only consistent risk factors associated with having any resistance, MDR-TB, and complete first-line resistance. In line with our findings, most studies do not report any significant association between sex and MDR-TB [42, 43, 45, 56, 57]. Nevertheless, Faustini, Hall and Perucci [44] report male sex to be a risk factor, while Liu et al. [52] found an association between female sex and MDR-TB. Several other studies have found younger age to be associated with the development of MDR-TB [44, 46, 49, 52, 53]. We could not support these findings with the results from our multivariable analysis, but results from our descriptive analysis indicate a similar pattern.

Our study has a number of limitations. Our study is based on reported surveillance data and we did not have insight into the original laboratory results or into detailed DST information on the cases. Because our data includes a considerable number of migrant cases, who can be a mobile population group, our surveillance data is incomplete for a number of variables, especially on DST. Of the 48,044 notified active TB cases between 2008 and 2017 in Germany, 76.8% had DST results available for HRZES. Although this is above the European average,
notified DST testing rates in Germany do not meet ECDC's target for TB elimination [7]. According to the ECDC, performing cultures and DST in at least 80% of the cases is a necessary step to achieve the elimination of TB in Europe [58]. As a result, our lower DST testing coverage should be interpreted with caution. Moreover, DST for E and Z are less reliable than DST for H and R, especially in MDR-TB cases. Information on resistance against second-line drugs is limited and could therefore not be included in our analysis. Information on social determinants and behavioural risk factors such as HIV status, unemployment, alcohol and drug abuse, smoking, and diabetes is not reported at the national level of the notification system and could also not be included in the risk factor analysis. Since data on prior treatment was incomplete, we utilized prior TB diagnosis as proxy, but this may mean that we may have missed relevant information on the type of prior treatment and its outcome.

Conclusion

Drug resistance patterns observed in Germany, particularly for MDR-TB cases, are far more complex than expected. Although the overall proportions of drug-resistance and MDR-TB are low, most MDR-TB cases in Germany present with additional resistances against other standard anti-TB drugs and almost 40% of MDR cases showed complete resistance against all five standard TB drugs (HRZES). As drug resistance patterns vary significantly in different subgroups, our findings highlight the importance of considering demographic characteristics of cases and knowing the patients’ full drug resistance profile to tailor treatment for optimal outcome. This is especially significant as our study found higher than expected proportions of drug resistances and MDR-TB among children and German-born adults. Finally, our finding of considerably high H monoresistance rates is important towards informing LTBI treatment regimens in Germany.

Author Contributions

Conceptualization: Doris Altmann, Barbara Hauer, Walter Haas.

Data curation: Doris Altmann.

Formal analysis: Saskia Glasauer, Doris Altmann.

Methodology: Nita Perumal.

Supervision: Nita Perumal.

Writing – original draft: Saskia Glasauer.

Writing – review & editing: Doris Altmann, Barbara Hauer, Bonita Brodhun, Walter Haas, Nita Perumal.

References

1. World Health Organization. Global tuberculosis report 2018. Geneva: World Health Organization; 2018.
2. World Health Organisation. The End TB Strategy: World Health Organisation; 2014 [Available from: http://www.who.int/tb/strategy/end-tb/en/]
3. Lonnroth K, Migliori GB, Abubakar I, D'Ambrosio L, de Vries G, Diel R, et al. Towards tuberculosis elimination: an action framework for low-incidence countries. The European respiratory journal. 2015; 45 (4):928–52. https://doi.org/10.1183/09031936.00214014 PMID: 25792630
4. The review on antimicrobial resistance. Tackling drug-resistant infections globally: final report and recommendations 2016 [Available from: https://amr-review.org/sites/default/files/160525_Final%20paper_with%20cover.pdf]
5. van der Werf M, M S. Drug-resistance—a challenge for tuberculosis control in the European Union and European Economic Area. Euro Surveill. 2014; 19(11).
6. Abubakar I, Dara M, Manissero D, Zumla A. Tackling the spread of drug-resistant tuberculosis in Europe. Lancet (London, England). 2012; 379(9813):e21–3.
7. European Center for Disease Prevention and Control. Annual Epidemiological Report for 2016—Tuberculosis. Stockholm: ECDC; 2018.
8. Kódmön C, Hollo V, Hultric E, Amato-Gauci A, Manissero D. Multidrug- and extensively drug-resistant tuberculosis: a persistent problem in the European Union European Union and European Economic Area. Euro Surveill. 2010; 15(11).
9. World Health Organization. Basic facts on tuberculosis (TB) in the WHO European Region 2018 [Available from: http://www.euro.who.int/en/health-topics/communicable-diseases/tuberculosis/data-and-statistics.
10. European Center for Disease Prevention and Control, WHO Regional Office for Europe. Tuberculosis surveillance and monitoring in Europe 2018–2016 data. Stockholm: European Center for Disease Prevention and Control; 2018.
11. de Beer J, Kódmön C, van der Werf M, van Ingen J, van Soolingen D, participants. TEM-Tms p. Molecular surveillance of multidrug- and extensively drugresistant tuberculosis transmission in the European Union from 2003 to 2011. Euro Surveill. 2014; 19(11).
12. European Center for Disease Prevention and Control, WHO Regional Office for Europe. Tuberculosis surveillance and monitoring in Europe. Stockholm: European Center for Disease Prevention and Control; 2014.
13. Ruesen C, van Gageldonk-Lafeber AB, de Vries G, Erkens CG, van Rest CG, Horthals Altes H, et al. Extent and origin of resistance to antituberculosis drugs in the Netherlands, 1993 to 2011. Euro Surveill. 2014; 19(11).
14. Somoskovi A, Helbling P, Degjim V, Hörnke R, Ritter C, EC B. Transmission of multidrug-resistant tuberculosis in a low-incidence setting, Switzerland, 2006 to 2012. Euro Surveill. 2012; 19(11).
15. Robert Koch Institut. Bericht zur Epidemiologie der Tuberkulose in Deutschland für 2017. Berlin: Robert Koch Institut; 2018.
16. van der Werf MJ, Kodmorn C, Hollo V, Sandgren A, Zucs P. Drug resistance among tuberculosis cases in the European Union and European Economic Area, 2007 to 2012. Euro Surveill. 2014; 19(10).
17. Caminero J. ed. Guidelines for Clinical and Operational Management of Drug-Resistant Tuberculosis. Paris: International Union Against Tuberculosis and Lung Disease; 2013.
18. World Health Organization. WHO treatment guidelines for drug-resistant tuberculosis. Geneva: World Health Organization; 2016.
19. Schaberg T, Bauer T, Brinkmann F, Diel R, Feiterna-Sperling C, Haas W, et al. S2k-Leitlinie: Tuberkulose im Erwachsenenalter. Pneumologie. 2017; 71:325–97. https://doi.org/10.1055/s-0043-105954 PMID: 28651293
20. Santos A, Leung J, Malaquias T, Vieira MAMDS, Kritski A, Mello FCQ. The Reliability of Rifampicin Resistance Identified on Xpert MTB/RIF as a Proxy for Multidrug-Resistant Tuberculosis (MDR-TB) in a Reference Center for MDR-TB in Rio de Janeiro, Brazil Am J Respir Crit Care Med. 2018; 197(A5544).
21. Boakye-Appiah J, Steinmetz A, Pupulampu P, Ofori-Yirenkyi S, Teteh I, Frimping M, et al. High prevalence of multidrug-resistant tuberculosis among patients with rifampicin resistance using GeneXpert Mycobacterium tuberculosis/rifampicin in Ghana. Int J Mycobacteriol. 2016; 5(3):226–30.
22. Huang H, Zhang Y, Li S, Wang J, Chen J, Pan Z, Gan H., Rifampicin Resistance and Multidrug-Resistant Tuberculosis Detection Using Xpert MTB/RIF in Wuhan, China: A Retrospective Study. Microb Drug Resist. 2018; 24(5).
23. World Health Organisation. Using the Xpert MTB/RIF assay to detect pulmonary and extrapulmonary tuberculosis and rifampicin resistance in adults and children. Geneva: World Health Organization; 2013.
24. Nasiri M, Zamani S, Pormohammad A, Feizabadi MM, Aslani HR, Amin M, et al. The reliability of rifampicin resistance as a proxy for multidrug-resistant tuberculosis: a systematic review of studies from Iran. Eur J Clin Microbiol Infect Dis. 2018; 37(1):9–14. https://doi.org/10.1007/s10096-017-3079-4 PMID: 28825010
25. Smith S, Kurbatova EV, Cavanaugh J, JP C, Global isoniazid resistance patterns in rifampicin-resistant and rifampin-susceptible tuberculosis. Int J Tuberc Lung Dis. 2011; 16(2):203–5.
26. Asaad AM, Alqahtani JM. Primary anti-tuberculious drugs resistance of pulmonary tuberculosis in Southwestern Saudi Arabia. J Infect Public Health. 2012; 5(4):281–5. https://doi.org/10.1016/j.jiph.2012.03.005 PMID: 23021650
27. Shamaei M, Marjani M, Chitsaz E, Kazempour M, Esmaeilii M, Farnia P, et al. First-line anti-tuberculosis drug resistance patterns and trends at the national TB referral center in Iran—eight years of surveillance. Int J Infect Dis. 2009; 13(5):e236–40. https://doi.org/10.1016/j.ijid.2008.11.027 PMID: 19285897

28. Cain KP, Benoit SR, Winston CA, Mac Kenzie WR. Tuberculosis among foreign-born persons in the United States. Jama. 2008; 300(4):405–12. https://doi.org/10.1001/jama.300.4.405 PMID: 18647983

29. Paramasivnan CN, Rehman F, Wares F, Sundar Mohan N, Sundar S, Devi S, et al. First- and second-line drug resistance patterns among previously treated tuberculosis patients in India. Int J Tuberc Lung Dis. 2010; 14(2):243–6. PMID: 20074419

30. He XC, Zhang XX, Zhao JN, Liu Y, Yu CB, Yang GR, et al. Epidemiological Trends of Drug-Resistant Tuberculosis in China From 2007 to 2014: A Retrospective Study. Medicine (Baltimore). 2016; 95(15): e3336.

31. Mesfin EA, Beyene D, Tesfaye A, Admasu A, Addise D, Amare M, et al. Drug-resistance patterns of Mycobacterium tuberculosis strains and associated risk factors among multi drug-resistant tuberculosis suspected patients from Ethiopia. PLoS One. 2018; 13(6):e0197737. https://doi.org/10.1371/journal.pone.0197737 PMID: 29864118

32. Bundesamt Deutscher. Gesetz zur Modernisierung der epidemiologischen Überwachung übertragbarer Krankheiten. Bonn: Bundesgesetzblatt; 2017.

33. Faensen D, Claus H, Benzel J, Ammon A, Pfoch T, Breuer T, et al. SurvNet@RKi—a multistate electronic reporting system for communicable diseases. Euro Surveill. 2006; 11(4):100–3. PMID: 16645245

34. Robert Koch Institut. Leitfaden zur Übermittlung von Fallberichten zur Tuberkulose. Berlin: Robert Koch Institut, 2004.

35. Hoffner S. Unexpected high levels of multidrug-resistant tuberculosis present new challenges for tuberculosis control. Lancet (London, England). 2012; 380(9851):1367–9.

36. Drobniowski F, Rüsch-Gerdes S, Hoffner S, Subcommittee on Antimicrobial Susceptibility Testing of Mycobacterium tuberculosis of the European Committee for Antimicrobial Susceptibility Testing of the European Society of Clinical Microbiology and Infectious Disease. Antimicrobial Susceptibility Testing of Mycobacterium Tuberculosis (EUCAST document E.DEF 8.1)—Report of the Subcommittee on Antimicrobial Susceptibility Testing of Mycobacterium tuberculosis of the European Committee for Antimicrobial Susceptibility Testing (EUCAST) of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID). Clin Microbiol Infect. 2007; 13(12):1144–56. https://doi.org/10.1111/j.1469-0691.2007.01813.x PMID: 17727670

37. Pierre-Audigier C, Surcouf C, Cadet-Daniel V, Namouchi A, Heng S, Murray A, et al. Fluoroquinolone and pyrazinamide resistance in multidrug-resistant tuberculosis. Int J Tuberc Lung Dis. 2012; 16 (2):221–3. https://doi.org/10.5588/ijtld.11.0266 PMID: 22236923

38. Schaberg T, Bauer T, Castell S, Dalhoff K, Detjen A, Diel R, et al. Empfehlungen zur Therapie, Chemoprophylaxe der Tuberkulose im Erwachsenen- und Kindesalter. Pneumologie. 2012; 66:133–71. https://doi.org/10.1055/s-0031-1291619 PMID: 22328186

39. RStudio Team. RStudio: Integrated Development for R. Boston, MA: RStudio, Inc.; 2015.

40. Durr S, Muller B, Alonso S, Hattendorf J, Laisse CJ, van Helden PD, et al. Differences in primary sites of infection between zoonotic and human tuberculosis: results from a worldwide systematic review. PLoS Negl Trop Dis. 2013; 7(8):e2399. https://doi.org/10.1371/journal.pntd.0002399 PMID: 24009789

41. Gallivan M, Shah N, Flood J. Epidemiology of human Mycobacterium bovis disease, California, USA, 2003–2011. Emerg Infect Dis. 2015; 21(3):435–43. https://doi.org/10.3201/eid2103.141539 PMID: 25693687

42. Demile B, Amare Z, Shewaye H, Xia S, Guadie A. Risk factors associated with multidrug-resistant tuberculosis (MDR-TB) in a tertiary armed force referral and teaching hospital, Ethiopia. BMC Infect Dis. 2018; 18(249).

43. Desissa F, Workineh T, Beyene T. Risk factors for the occurrence of multi-drug resistant tuberculosis among patients undergoing multidrug-resistant tuberculosis treatment in East Shoa, Ethiopia. BMC Public Health. 2018; 18(422).

44. Faustini A, Hall A J, Perucci A. Risk factors for multidrug resistant tuberculosis in Europe: a systematic review. Thorax. 2006; 61:158–63. https://doi.org/10.1136/thx.2005.045963 PMID: 16254056

45. Gomes M, Correia A, Mendonça D, Duarte R. Risk Factors for Drug-Resistant Tuberculosis. Tuberc Res. 2014; 2:111–8.

46. Workicho A, Kassahun W, F A. Risk factors for multidrug-resistant tuberculosis among tuberculosis patients: a case-control study. Infect Drug Resist. 2017(10):91–6.

47. Hargreaves S, Lönnroth K, Nellums LB, Olaru ID, Nathavitharan RA, Norredam M, Friedland JS. Multi-drug-resistant tuberculosis and migration to Europe. Clin Microbiol Infect. 2017; 23:141–6. https://doi.org/10.1016/j.cmi.2016.09.009 PMID: 27665703
48. Papaventisis D, Nikolaou S, Karabela S, Ioannidis P, Konstantinidou E, Marinou I, et al. Tuberculosis in Greece: bacteriologically confirmed cases and antituberculosis drug resistance. Euro Surveill. 2010; 15(28).

49. Clark C, Li J, Driver R, Munsiff S. Risk factors for drug-resistant tuberculosis among non-US-born persons in New York City. Int J Tuberc Lung Dis. 2005; 9(9):964–9. PMID: 16158888

50. Falzon D, Infuso A, Alt-Belghiti F. In the European Union, TB patients from former Soviet countries have a high risk of multidrug resistance. Int J Tuberc Lung Dis. 2006; 10(9):954–8. PMID: 16964783

51. Lombardi G, Dal Monte P, Denicolò A, Tadolini M, Martelli G, Bacchi Reggiani ML, et al. Trend of microbiologically-confirmed tuberculosis in a low-incidence setting with high immigration rates. BMC Public Health. 2014; 14(340).

52. Liu Q, Zhu L, Shao Y, Song H, Li G, Zhou Y, et al. Rates and risk factors for drug resistance tuberculosis in Northeastern China. BMC Public Health. 2013; 13(1171).

53. Shao Y, Yang D, Xu W, Lu W, Song H, Dai Y, et al. Epidemiology of anti-tuberculosis drug resistance in a chinese population: current situation and challenges ahead. BMC Public Health. 2011; 11(110).

54. Walker TM, Merker M, Knoblach AM, Helbling P, Schoch OD, van der Werf MJ, et al. A cluster of multidrug-resistant Mycobacterium tuberculosis among patients arriving in Europe from the Horn of Africa: a molecular epidemiological study. Lancet Infect Dis. 2018; 18(4):431–40. https://doi.org/10.1016/S1473-3099(18)30004-5 PMID: 29326013

55. Eddabra R, Ait Benhassou H. Rapid molecular assays for detection of tuberculosis. Pneumonia (Nathan Qld). 2018; 10:4–.

56. Dessalegn M, Daniel E, Behailu S, Wagnew M, Nyagero J. Predictors of multidrug resistant tuberculosis among adult patients at Saint Peter Hospital Addis Ababa, Ethiopia. Pan Afr Med J. 2016; 25(Suppl 2):5. https://doi.org/10.11604/pamj.supp.2016.25.2.9203 PMID: 28439330

57. Gobena D, Ameya G, Haile K, Abreha G, Worku Y, Debela T. Predictor of multidrug resistant tuberculosis in southwestern part of Ethiopia: a case control study. Ann Clin Microbiol Antimicrob. 2018; 17(1):30. https://doi.org/10.1186/s12941-018-0283-8 PMID: 29970076

58. European Center for Disease Prevention and Control. Progressing towards TB elimination. Stockholm: European Center for Disease Prevention and Control; 2010.