Trends of multidrug-resistant tuberculosis clustering in Portugal

To the Editor:

Multidrug-resistant (MDR) tuberculosis (TB) represent a major threat for global TB control. In 2017, the World Health Organization estimated 460,000 cases of MDR-TB, of which 8.5% were also extensively drug-resistant (XDR) cases [1]. In Portugal, over the last decade, the decreasing tendency of TB cases is about 7% per year, and the proportion of MDR-TB cases remains steadily around 1% of the total TB cases. In 2017, the preliminary report of the Portuguese national TB programme reported 1607 new cases of pulmonary TB, with 12 MDR-TB cases [2].

Since 2014, there have been specific centres for the diagnosis, consultancy, monitoring and treatment of the MDR/XDR-TB cases. Besides providing a clinical approach, these centres also aim to monitor these resistant cases, linking the epidemiological survey performed within the community by public health authorities [3] and systematic molecular genotyping performed by the National Reference Laboratory (NRL). Since the Portuguese NRL receives all the strains isolated from all the MDR-TB patients from Portugal (mandatory since 2007) [4], this approach could allow a very good correlation between the genetic and epidemiological information in order to detect both the resistance profiles, as there are possible relationships between strains due to the occurrence of ongoing transmission [5, 6].

In this study, we intended to analyse the MDR-TB clustering rate in Portugal.

From a total of 78 MDR/XDR-TB strains identified and notified in the country during 2014–2017, 71 (91.0%) were available for molecular analysis. From these 78 strains, seven were not available for further analysis due to contamination of the culture or MDR diagnosis based only on molecular biology methodologies (GeneXpert or other line-probe assays). The drug susceptibility profiles are described in table 1.

For each strain, 24-loci MIRU-VNTR (mycobacterial interspersed repetitive units – variable number of tandem repeats) genotyping was performed by standardised protocols using a MIRU-VNTR typing kit, according to the manufacturer’s instructions (GenoScreen, Lille, France). Dendrograms were constructed using the online free software MIRU-VNTRplus (https://miru-vntrplus.org/MIRU/miruinfo.faces). A molecular cluster was defined whenever different strains shared the exact MIRU-VNTR profile. All clusters identified were further analysed with the available epidemiological data.

The majority of the MDR-TB cases were male (75.6%) with a median age of 44.3 years (minimum 15 and maximum 75 years). Most of these cases were notified in the Lisbon and Tagus Valley (LTV) region (64.0%) and the North region (23.1%). XDR-TB cases were identified in 15 cases (19.2%), of which 86.7% were from the LTV region (table 1).

Using MIRU-VNTR, seven different clusters were identified (table 1), ranging from two to 14 strains. Overall, the proportion of MDR-TB cases attributable to recent transmission in the study period (2014–2017), on the basis of genetic data, was 63.4% (45 out of 71).

From the analysis of the molecular data, we observed a decreasing tendency of the cases that can be potentially related to recent transmission. In fact, in 2014, we found six clusters ranging from two to four strains, corresponding to a clustering rate of 72.7%. The major cluster was from strains isolated in the...
| Lab no. | Diagnosis year | Exclusion reason | Sex | Age years | Region of isolation | MDR/ XDR-TB | STR | INH | RMP | EMB | PZA | AMI | CAP | ETI | MOX | OFL | LIN | KAN | CIC | PAS | Cluster no. |
|--------|----------------|------------------|-----|-----------|---------------------|-------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| S199228 | 2014 | M 42 | LTV | XDR | R | R | R | R | R | R | R | R | S | S | S | S | S | S | S | S | S | S | S | S |
| 2014 | No culture isolation | | M 43 | LTV | XDR | R | R | R | R | R | R | R | R | S | S | S | S | S | S | S | S | S | S | S |
| TB25429 | 2014 | M 47 | LTV | MDR | NA | R | R | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| P1595 | 2014 | M 37 | LTV | MDR | R | R | R | R | R | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S |
| P1279 | 2014 | M 53 | LTV | MDR | R | R | R | R | R | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S |
| S20779 | 2014 | M 48 | LTV | MDR | R | R | R | R | R | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S |
| P1378 | 2014 | M 50 | LTV | XDR | R | R | R | R | R | S | S | S | R | R | R | S | S | S | S | S | S | S | S | S |
| P163 | 2014 | M 53 | LTV | XDR | R | R | R | R | R | S | S | S | R | R | R | R | R | R | R | R | R | R | R | R |
| TB24818 | 2014 | F 32 | LTV | MDR | R | R | R | R | R | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S |
| P291 | 2014 | M 28 | LTV | MDR | R | R | R | R | R | S | S | S | R | R | R | R | R | R | R | R | R | R | R | R |
| TB25274 | 2014 | M 37 | LTV | XDR | R | R | R | R | R | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S |
| S211891 | 2014 | M 36 | LTV | MDR | R | R | R | R | R | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S |
| P1599 | 2014 | M 43 | LTV | MDR | R | R | R | R | R | S | S | S | S | S | S | S | S | S | S | S | S | S | S |
| P187 | 2014 | M 58 | LTV | MDR | R | R | R | R | R | S | S | S | S | S | S | S | S | S | S | S | S | S | S |
| P88 | 2014 | M 63 | North | MDR | R | R | R | R | R | S | S | S | S | S | S | S | S | S | S | S | S | S | S |
| P2423 | 2014 | F 75 | North | MDR | R | R | R | R | R | S | S | S | S | S | S | S | S | S | S | S | S | S | S |
| P292 | 2014 | F 41 | North | MDR | R | R | R | R | R | S | S | S | S | S | S | S | S | S | S | S | S | S | S |
| P1536 | 2014 | M 23 | North | MDR | R | R | R | R | R | S | S | S | S | S | S | S | S | S | S | S | S | S |
| P1536 | 2014 | F 47 | North | MDR | R | R | R | R | R | S | S | S | S | S | S | S | S | S | S | S | S | S |
| P356 | 2014 | M 42 | North | MDR | S | R | R | S | S | S | S | S | S | S | S | S | S | S | S | S | S |
| P92 | 2014 | M 59 | Centre | MDR | R | R | R | S | S | S | S | S | S | S | S | S | S | S | S | S | S |
| P340 | 2015 | M 48 | Centre | XDR | R | R | R | R | R | S | S | S | R | S | S | S | S | S | S | S | S | S |
| 2015 | Only LPA | | M 31 | LTV | MDR | R | R | R | R | R | S | S | S | S | R | S | S | S | S | S | S | S |
| P2624 | 2015 | M 28 | LTV | MDR | R | R | R | R | R | S | S | S | R | S | S | S | S | S | S | S | S | S |
| P1928 | 2015 | M 61 | LTV | MDR | R | R | R | R | R | S | S | S | S | S | S | S | S | S | S | S |
| P1229 | 2015 | M 39 | LTV | MDR | R | R | R | R | R | R | R | R | R | R | S | S | S | S | S | S |
| P1876 | 2015 | M 55 | LTV | XDR | R | R | R | R | R | R | R | R | R | R | S | S | S | S | S | S |
| P1926 | 2015 | M 52 | LTV | XDR | R | R | R | R | R | R | R | R | R | R | R | S | S | S | S | S | S |
| P2829 | 2015 | M 44 | LTV | MDR | R | R | R | R | R | S | S | S | S | S | S | S | S | S | S | S |
| P2184 | 2015 | M 75 | LTV | MDR | R | R | R | R | R | R | R | R | R | R | R | R | R | R | S | S |
| P1994 | 2015 | M 41 | LTV | MDR | R | R | R | R | R | S | S | S | S | S | S | S | S | S | S |
| P2058 | 2015 | M 57 | LTV | MDR | R | R | R | R | R | S | S | S | S | S | S | S | S | S | S |
| TB24737 | 2015 | M 44 | LTV | MDR | R | R | R | R | R | R | R | R | R | R | R | R | R | R | S | S |
| P1585 | 2015 | F 42 | LTV | MDR | R | R | R | R | R | S | S | S | S | S | S | S | S | S | S |
| P2354 | 2015 | F 27 | LTV | MDR | R | R | R | R | R | S | S | S | S | S | S | S | S | S | S |
| P2471 | 2015 | M 34 | LTV | MDR | R | R | R | R | R | S | S | S | S | S | S | S | S | S | S |
| 2015 | Only LPA | | F 32 | LTV | MDR | NA | R | R | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| P884 | 2015 | M 61 | LTV | MDR | R | R | R | R | R | S | S | S | S | S | S | S | S | S | S | S | S |
| P2579 | 2015 | M 35 | LTV | MDR | R | R | R | R | R | S | S | S | S | S | S | S | S | S | S | S |
| P2452 | 2015 | M 57 | North | MDR | R | R | R | R | R | S | S | S | S | S | S | S | S | S | S |
| P982 | 2015 | M 37 | North | MDR | R | R | R | R | R | S | S | S | S | S | S | S | S | S | S |

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| Lab no.   | Diagnosis year | Exclusion reason | Sex | Age years | Region of isolation | MDR/ XDR-TB | STR | INH | RMP | EMB | PZA | AMI | CAP | ETI | MOX | OFL | LIN | KAN | CIC | PAS | Cluster no. |
|-----------|----------------|------------------|-----|-----------|--------------------|--------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| P1880     | 2015           |                  | M   | 58        | North              | MDR          | R   | R   | R   | R   | S   | S   | S   | S   | S   | S   | S   | S   | R   | S   | S   | S   | 5   |
| P2353     | 2015           |                  | M   | 21        | Island of Madeira | MDR          | S   | R   | R   | R   | S   | S   | S   | S   | R   | S   | S   | S   | S   | S   | S   | S   | 5   |
| P27       | 2015           | Only LPA         | M   | 44        | Algarve            | MDR          | S   | R   | R   | R   | S   | S   | S   | S   | R   | S   | S   | S   | S   | S   | S   | S   | 5   |
| S308144   | 2016           |                  | M   | 41        | LTV                | XDR          | R   | R   | R   | R   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | 3   |
| S309948   | 2016           |                  | F   | 41        | North              | MDR          | R   | R   | R   | R   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | 4   |
| S310368   | 2016           |                  | M   | 42        | North              | MDR          | R   | R   | R   | R   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | 3   |
| S312205   | 2016           |                  | F   | 63        | LTV                | MDR          | R   | R   | R   | R   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | 1   |
| S314371   | 2016           |                  | M   | 15        | LTV                | MDR          | R   | R   | R   | R   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | 6   |
| S324134   | 2016           | No culture isolation | F   | 45        | LTV                | MDR          | R   | R   | R   | R   | S   | S   | S   | S   | S   | S   | S   | S   | 6   |
| S326551   | 2016           |                  | M   | 40        | LTV                | MDR          | R   | R   | R   | R   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | 3   |
| S327889   | 2016           |                  | F   | 22        | LTV                | MDR          | R   | R   | R   | R   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | 3   |
| S320857   | 2016           |                  | M   | 43        | North              | MDR          | R   | R   | R   | R   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | 3   |
| S316569   | 2016           |                  | M   | 54        | North              | MDR          | R   | R   | S   | R   | S   | R   | S   | S   | S   | S   | S   | S   | S   | S   | 3   |
| S326782   | 2016           |                  | M   | 62        | North              | MDR          | R   | R   | R   | S   | S   | S   | S   | R   | S   | S   | S   | S   | S   | S   | 3   |
| S340248   | 2016           |                  | M   | 20        | LTV                | MDR          | R   | R   | R   | R   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | 2   |
| TB31393   | 2016           |                  | F   | 40        | LTV                | MDR          | R   | R   | R   | R   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | 3   |
| S332846   | 2016           |                  | M   | 70        | Centre             | MDR          | R   | R   | R   | R   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | 3   |
| S347401   | 2016           |                  | F   | 20        | LTV                | MDR          | R   | R   | R   | R   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | 2   |
| ACC       | 2016           |                  | F   | 40        | LTV                | MDR          | R   | R   | R   | R   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | 2   |
| S333605   | 2016           |                  | M   | 62        | North              | MDR          | R   | R   | R   | R   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | 3   |
| S348387   | 2016           |                  | M   | 44        | UNK                | MDR          | R   | R   | R   | R   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | 3   |
| S352139   | 2017           |                  | F   | 22        | Centre             | MDR          | R   | R   | R   | R   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | 6   |
| S375001   | 2017           |                  | F   | 22        | Centre             | MDR          | R   | R   | R   | R   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | 5   |
| S374668   | 2017           |                  | F   | 34        | LTV                | MDR          | R   | R   | R   | R   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | 5   |
| S381277   | 2017           |                  | M   | 30        | North              | MDR          | R   | R   | R   | R   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | 5   |
| TB33470   | 2017           |                  | M   | 52        | LTV                | MDR          | R   | R   | R   | R   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | 1   |
| S389865   | 2017           |                  | F   | 59        | LTV                | MDR          | R   | R   | R   | R   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | 1   |
| S387683   | 2017           |                  | M   | 56        | LTV                | XDR          | R   | R   | R   | R   | S   | S   | S   | S   | R   | R   | S   | S   | S   | S   | 3   |
| S399045   | 2017           |                  | F   | 41        | North              | MDR          | R   | R   | R   | R   | S   | S   | S   | S   | R   | R   | S   | S   | S   | S   | 3   |
| S393977   | 2017           |                  | M   | 51        | LTV                | XDR          | R   | R   | R   | R   | S   | S   | S   | S   | R   | R   | S   | S   | S   | S   | 1   |
| S39986    | 2017           |                  | M   | 58        | LTV                | MDR          | R   | R   | R   | R   | S   | S   | S   | S   | R   | R   | S   | S   | S   | S   | 3   |
| TB34192   | 2017           |                  | M   | 62        | LTV                | MDR          | R   | R   | R   | R   | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   | 2   |

MDR: multidrug-resistant; XDR: extensively drug-resistant; TB: tuberculosis; STR: streptomycin; INH: isoniazid; RMP: rifampicin; EMB: ethambutol; PZA: pyrazinamide; AMI: amikacin; CAP: capreomycin; ETI: ethionamide; MOX: moxifloxacin; OFL: ofloxacin; LIN: linezolid; KAN: kanamycin; CIC: cycloserine; PAS: para-aminosalicylic acid; M: male; F: female; LTV: Lisbon and Tagus Valley; R: resistant; S: sensitive; NA: not available; LPA: line-probe assay; UNK: unknown.
North region and the remaining clusters were mainly from LTV strains. Regarding the MDR-TB strains isolated in 2015, three clusters were found, with a clustering rate of 55.0%, ranging from two to five strains. All the clustered strains were from the LTV region with the exception of one strain that belonged to a patient from the North region. In 2016, only two clusters were found with two and five strains, with a clustering rate of 38.9%. The minor cluster was from a mother/child and the larger included strains from the LTV and the North regions. Finally, in 2017, two clusters were identified with two strains each, all from the LTV region, corresponding to a clustering rate of 36.4%.

When linking the epidemiological and the molecular data, we did not find a good agreement. After adjustment for confirmed epidemiological links, the overall cluster rate (2014–2017) decreased from 63.4% to 14.9%.

This study has a limitation related to the possible heterogeneity of the epidemiological enquiries. However, it has the strength of collecting all MDR-TB samples in the country for 4 years to be analysed in the NRL. We observed, in the studied period, a decreasing tendency both in the number of MDR-TB cases and the clustering rates, despite a poor agreement between laboratory and epidemiological data. The centralisation of the MDR-TB cases in reference centres seems to be effective, although there is a need for a better molecular tool, with higher discriminatory power, and better inclusion of epidemiological data when discussing these clusters.

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