ORIGINAL RESEARCH LETTER

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-VNTR*plus* (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

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Analysis of MDR-TB rates showed consistent decreases in cases and clustering rates but did not establish valid relationships with the epidemiological information collected by the public health authorities http://ow.ly/3BnN30nxqW3

Cite this article as: Macedo R, Duarte R. Trends of multidrug-resistant tuberculosis clustering in Portugal. *ERJ Open Res* 2019; 5: 00151-2018 [https://doi.org/10.1183/23120541.00151-2018].

TABLE 1 Microbiological and demographic characteristics of the patients enrolled in the study

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	R	R	S	S	S	S	
	2014	No culture isolation	M	43	LTV	XDR	R	R	R	R	R	R	R	R	R	R	S	S	S	S	
	2014	Only LPA	M	47	LTV	MDR	NA	R	R	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
TB25429	2014		M	41	LTV	MDR	R	R	R	R	R	S	S	R	S	S	S	S	S	S	6
P1595	2014		M	37	LTV	MDR	R	R	R	S	R	S	S	R	S	S	S	S	S	S	1
P1279	2014		F	31	LTV	MDR	R	R	R	R	R	S	S	R	S	S	S	R	S	S	
S207797	2014		M	53	LTV	MDR	R	R	R	S	R	S	S	R	S	S	S	S	S	S	2
P1428	2014		M	48	LTV	MDR	R	R	R	S	R	S	S	R	S	S	S	S	S	S	1
P1378	2014		M	50	LTV	XDR	R	R	R	R	R	S	S	R	R	R	S	R	S	S	3
P163	2014		M	53	LTV	XDR	R	R	R	R	R	R	S	R	R	R	S	R	S	S	3
TB24818	2014		F	32	LTV	MDR	R	R	R	R	S	S	S	S	S	S	S	S	S	S	7
P291	2014		M	28	LTV	MDR	R	R	R	R	S	S	S	S	S	S	S	S	S	S	
TB25274	2014		M	37	LTV	XDR	R	R	R	R	R	S	S	R	S	R	S	R	R	S	
S211891	2014		M	36	LTV	MDR	R	R	R	R	S	S	S	R	S	S	S	S	S	S	7
P1599	2014		M	43	LTV	MDR	R	R	R	R	S	S	S	S	S	S	S	S	S	S	6
P187	2014		M	58	LTV	MDR	R	R	R	R	R	S	S	R	S	S	S	S	S	S	2
P88	2014		M	63	North	MDR	R	R	R	S	S	S	S	S	S	S	S	S	S	S	4
P423	2014		F	75	North	MDR	R	R	R	S	S	S	S	S	S	S	S	S	S	S	4
P292	2014		F	41	North	MDR	R	R	R	S	S	S	S	S	S	S	S	S	S	S	4
P1536	2014		M	23	North	MDR	R	R	R	S	S	S	S	S	S	S	S	S	S	S	4
P729	2014		M	47	North	MDR	R	R	R	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	
P92	2014		M	59	Centre	MDR	R	R	R	S	S	S	S	R	S	S	S	S	S	S	2
P340	2014		M	48	Centre	XDR	R	R	R	R	S	R	S	R	S	R	S	R	S	S	3
	2015	Only LPA	M	31	LTV	MDR	NA	R	R	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
P2624	2015		M	28	LTV	MDR	R	R	R	R	S	S	S	R	S	S	S	S	S	S	7
P1928	2015		M	61	LTV	MDR	R	R	R	R	R	S	S	R	S	S	S	S	S	S	1
P1229	2015		M	39	LTV	XDR	R	R	R	R	R	R	R	R	R	R	S	R	S	S	1
P1876	2015		M	55	LTV	XDR	R	R	R	R	R	R	S	R	R	R	S	R	S	S	3
P1926	2015		M	52	LTV	XDR	R	R	R	R	R	R	R	R	R	R	S	R	S	S	1
P2829	2015		M	44	LTV	MDR	S	R	R	S	S	S	S	R	S	S	S	S	S	S	1
P2184	2015		M	75	LTV	MDR	R	R	R	R	R	R	R	R	S	S	S	R	S	S	1
P1994	2015		M	41	LTV	MDR	R	R	R	S	R	S	S	R	S	S	S	S	S	S	2
P2058	2015		M	57	LTV	MDR	R	R	R	S	R	S	S	R	S	S	S	S	S	S	
TB24737	2015		M	44	LTV	XDR	R	R	R	R	R	R	R	S	R	R	S	R	S	R	
P1585	2015		F	42	LTV	MDR	R	R	R	R	S	S	S	R	S	S	S	S	S	S	3
P2354	2015		F	27	LTV	MDR	R	R	R	R	R	R	R	S	S	S	S	R	S	S	
P2471	2015		M	34	LTV	MDR	R	R	R	R	S	S	S	R	S	S	S	S	S	S	3
	2015	Only LPA	F	32	LTV	MDR	NA	R	R	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
P884	2015		M	61	LTV	MDR	R	R	R	S	R	S	S	R	S	S	S	S	S	S	2
P2579	2015		M	35	LTV	MDR	R	R	R	R	R	S	S	R	S	S	S	S	S	S	
P2452	2015		M	57	North	MDR	R	R	R	S	S	S	S	R	S	S	S	S	S	S	3
P982	2015		M	37	North	MDR	R	R	R	R	R	S	S	S	S	S	S	S	S	S	

Continued

TABLE 1 Continued

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	S	S	S	S	S	S	S	S	R	S	S	
P2353	2015		M	21	Island of Madeira	MDR	S	R	R	R	S	S	S	R	S	S	S	S	S	S	5
P27	2015		M	44	Algarve	MDR	S	R	R	S	S	S	S	R	S	S	S	S	S	S	
	2015	Only LPA	M	39	Alentejo	MDR	NA	R	R	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
S308144	2016		M	41	LTV	XDR	R	R	R	R	R	R	S	R	R	R	S	R	S	S	3
S309968	2016		F	41	North	MDR	R	R	R	S	S	S	S	S	S	S	S	S	S	S	4
S310368	2016		M	42	North	XDR	R	R	R	R	R	R	S	R	R	R	S	R	S	S	3
S312205	2016		F	63	LTV	MDR	S	R	R	S	R	R	R	R	S	S	S	R	S	S	1
S314371	2016		M	15	LTV	MDR	R	R	R	R	S	S	S	R	S	S	S	S	S	S	6
S324134	2016		M	61	Centre	MDR	R	R	R	R	R	S	S	S	S	S	S	R	S	S	
	2016	No culture isolation	F	45	LTV	MDR	R	R	R	R	R	NA	NA	NA	NA	NA	NA	NA	NA	NA	
S326551	2016		M	40	LTV	MDR	R	R	R	R	R	S	S	S	S	S	S	S	S	S	
S327889	2016		F	22	LTV	XDR	R	R	R	R	R	R	S	R	R	R	S	R	S	S	3
S320857	2016		M	43	LTV	MDR	R	R	R	R	R	S	S	S	S	S	S	S	S	S	
S316569	2016		M	54	North	MDR	R	R	R	S	S	R	R	S	S	S	S	R	S	S	
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	S	R	S	S	R	S	S	S	S	S	S	2
TB31393	2016		F	40	LTV	MDR	R	R	R	S	S	S	S	S	S	S	S	S	S	S	
S332846	2016		M	70	Centre	MDR	R	R	R	S	R	S	S	S	S	S	S	S	S	S	
S347401	2016		F	20	LTV	MDR	R	R	R	R	S	S	S	R	S	S	S	S	S	S	
ACC	2016		F	40	LTV	MDR	R	R	R	S	R	S	S	R	S	S	S	S	S	S	2
S333605	2016		M	62	North	MDR	R	R	R	S	S	S	S	R	S	S	S	S	S	S	3
S348387	2016		M	UNK	Centre	MDR	S	R	R	S	S	S	S	S	S	S	S	S	S	S	
S352139	2017		F	22	Centre	MDR	R	R	R	R	R	S	S	S	S	S	S	S	S	S	6
S375001	2017		F	22	Centre	MDR	S	R	R	R	S	S	S	R	S	S	S	S	S	S	5
S374686	2017		F	34	LTV	MDR	S	R	R	R	R	S	S	R	S	S	S	S	S	S	
S381277	2017		M	30	North	MDR	R	R	R	R	S	S	S	S	S	S	S	S	S	S	
TB33470	2017		M	52	LTV	MDR	S	R	R	R	R	S	S	R	S	S	S	S	S	S	1
S389865	2017		F	59	North	MDR	R	R	R	R	R	S	S	S	S	S	S	S	S	S	
S387683	2017		M	56	LTV	XDR	R	R	R	R	R	R	S	R	R	R	S	R	S	S	3
S399045	2017		F	41	North	MDR	R	R	R	S	S	S	S	R	R	R	S	S	S	S	
S396397	2017		M	51	LTV	XDR	R	R	R	R	R	R	R	R	S	S	S	R	S	S	1
S399986	2017		M	58	LTV	MDR	R	R	R	R	S	S	S	R	R	R	S	S	S	S	3
TB34192	2017		M	62	LTV	MDR	R	R	R	S	S	S	S	R	S	S	S	S	S	S	2
	2017	Only LPA	M	54	North	MDR	NA	R	R	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	

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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Received: Sept 07 2018 | Accepted after revision: Jan 02 2019

Conflict of interest: None declared.

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