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A tale of two settings: the role of the Beijing genotype in the epidemiology of MDR-TB.

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Take home message for social media: Contrasting relationship between the Beijing genotype of *Mycobacterium tuberculosis* and MDR-TB in England versus Peru.

To the Editor:

Described as a 'template for success' by Hanekom *et al.*, the Beijing genotype of *Mycobacterium tuberculosis* (*M. tb*) has been associated with hypervirulence, drug resistance, evasion of the Bacillus Calmette-Guérin (BCG) vaccine and differential immunoregulation [1]. The genotype is itself diverse; accordingly the fact that specific traits have been associated with Beijing only in certain settings may be explained by variation in the sub-types that predominate in each.

We sought to explore the role that the Beijing genotype has played in the epidemiology of multi-drug resistant tuberculosis (MDR-TB) in two very different epidemiological settings: England and Peru. In the UK nearly three quarters of TB disease occurs amongst migrants, which may result from latent *M. tb* infections acquired from high-burden countries of origin. Thus the distribution of lineages in the UK reflects a composite of the circulating strains in other nations. Whilst increasing levels of MDR-TB in the UK have triggered understandable concern, only a small fraction of cases are MDR (2011: 1.0%) [2]. In contrast, the Peruvian TB epidemic is relatively 'home grown' and the prevalence of drug resistance is greater (2011: 5.3% MDR) [2]. Our objectives were thus to 1) identify any association between the Beijing genotype and MDR in these two settings and 2) to examine if trends in Beijing might explain changes in the prevalence of MDR-TB over time.

The following datasets were used during this study:

a) English TB cases:

Taken from Public Health England's Enhanced Tuberculosis Surveillance System and Mycobacterial Surveillance Network.

- England 1- 8,859 individuals diagnosed and 24-loci Mycobacterial Interspersed Repetitive Units-Variable Number Tandem Repeats (MIRU-VNTR) strain typed between 2010 and 2011. Of these, 4,661 had at least first line drug sensitivity data, a minimal 12-loci pre-defined profile [3] and were pulmonary.
- England 2- 189 MDR-TB cases from 2004-07 which had undergone 24-loci typing. 117 were pulmonary and had the minimal profile. 24-loci typing was not available for non-MDR cases from this period.

Susceptibility to rifampicin, isoniazid, ethambutol, pyrazinamide, streptomycin, amikacin, kanamycin, capreomycin, azithromycin, clarithromycin, ciprofloxacin, ofloxacin, moxifloxacin, prothionamide, ethionamide, cycloserine, 4-amino salicylic acid, linezolid, clofazamine and rifabutin had been tested using the proportion or resistance ratio methods.

b) Peruvian TB cases:

- Peru 1- 323 sputum positive cases of pulmonary TB recruited from clinical facilities in Lima between 2004 and 2006 extracted from Taype *et al.* [4]. 229 had been tested for sensitivity to rifampicin, isoniazid, ethambutol and streptomycin, of which 101 (44.1%) were MDR; we note that this figure is much higher than the overall proportion MDR in this setting [2], suggesting that drug susceptibility testing was prioritised for those at highest risk of MDR, consistent with previous Peruvian testing policies. All cases had a complete standard 12-loci profile.
- Peru 2- Database of 230 cases of pulmonary MDR-TB identified in Lima between 1996 and 2004 [5,6]. Isolates underwent drug susceptibility testing to rifampicin, isoniazid, ethambutol, pyrazinamide, streptomycin, kanamycin, capreomycin, ethionamide, 4-amino salicylic acid and levofloxacin. One individual lacking a complete standard 12-loci profile was excluded, leaving 229.

MIRU-VNTRplus was utilised to designate lineages for all four datasets to ensure comparability [7]. Substantial differences in the lineage and sublineage profiles of English (England 1, 4,661 individuals) and Peruvian (Peru 1, 229 individuals) pulmonary TB cases were apparent (χ^2 test of independence p-value <0.001). The vast majority of Peruvian cases were of the Euro-American lineage (92.6%; 212/229), with LAM the majority sublineage (48.1%; 102/212). The remainder of cases were nearly all Beijing (6.6%; 15/229). In contrast, English cases comprised a variety of lineages consistent with their multinational origins: 51.8% Euro-American (2,412/4,661), 28.6% Delhi-CAS (1,335/4,661), 9.2% EAI (429/4,661), 8.3% Beijing (392/4,661). The Euro-American sublineages seen were also diverse.

Restricting our analysis to MDR cases again revealed very different profiles between Peru and England (p-value <0.001). Peruvian cases (Peru 2, 229 individuals) were dominated by the Euro-American lineage (93.9%; 215/229), 56.3% of which was the LAM sublineage (121/215). Regression modelling of Peru 1 to explore the relationship between MDR and *M. tb* lineage showed no association (Beijing crude odds ratio (OR) 1.10 (95% confidence interval (CI) 0.38-3.14), Wald p-value 0.86; Euro-American baseline). In stark contrast to the overall distribution of lineages, among English MDR cases (England 1) the proportion Beijing increased to 47.4% (36/76). Regression modelling revealed a strong positive association between Beijing and MDR among English pulmonary cases (crude OR 11.51 (6.65-19.95), <0.005; adjusted OR 9.53 (4.87-18.65), <0.005; Euro-American baseline; England 1; models excluded non-*M. tb* cases and those with missing data). Age, gender, a composite social risk factor variable (homelessness, imprisonment, drug/alcohol abuse), country of birth, ethnic

group and previous diagnosis were adjusted for in the latter; this could not be done for Peru 1 as these variables were not available.

We also tested whether Beijing was enriched among strains with resistance to a greater number of drugs (figure 1a). Pre-MDR-TB (pre-MDR) was defined as resistance to either, but not both, isoniazid and rifampicin. Pre-extensively drug resistant (pre-XDR) cases were MDR plus resistant to either a fluoroquinolone or a second-line injectable. Beijing was enriched from pre-MDR to XDR cases in the English data (Pearson's correlation coefficient p_{trend} Beijing versus non-Beijing <0.005; England 1). This could be specifically observed among non-UK born, but not UK born, cases (data not shown). No trend was seen in the Peruvian data (p_{trend} 0.76; Peru 1 and Peru 2). Further analysis to determine whether the association has changed over time indicated a recent increase in the proportion of cases caused by Beijing (p_{trend} <0.005) (figure 1b; England 1 and England 2).

Our analysis suggests that the Beijing genotype plays a different role in the current epidemiology of MDR-TB in England and Peru. In England, the observed association likely reflects rising importation of resistant strains, rather than the development of resistance within the UK. In Peru there is likely to be a similarly high prevalence of MDR among all genotypes, potentially explaining why we did not observe an association. Interestingly, a recent paper by Iwamoto *et al.* suggests that the overall prevalence of Beijing has increased in Peru over the last decade, but the proportion of Beijing strains that are MDR has decreased [8].

We utilised available datasets from studies undertaken at different times with variable designs, thus some interpretative caution is required. For example, Peru 2 recruited from households with more than one pulmonary MDR cases, and thus could have oversampled clustered cases. However, the similarity of the lineage profiles of Peru 2 with Peru 1, which was not collected through such means, suggests this effect is limited. English cases were tested for resistance to a wider range of drugs than the Peruvian cases, thus the 'no known resistance' category is not strictly comparable between the two datasets. Nevertheless, only 1.3% of England 1 was resistant to drugs not tested within Peru 1.

Molecular epidemiological surveillance and addressing and preventing resistance have been highlighted as vital for TB elimination by the European Respiratory Society [9,10]. We demonstrate within this study how the tools of the former could be vital to aid the latter: within Peru where the MDR epidemic could worsen should fitter drug resistant Beijing strains evolve or enter the country, and within the UK where the lineage profile is highly dynamic.

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FOOTNOTES

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REFERENCES

1. Hanekom M, Gey van Pittius NC, McEvoy C, Victor TC, Van Helden PD, Warren RM. Mycobacterium tuberculosis Beijing genotype: a template for success. *Tuberculosis (Edinb)* 2011;91(6):510-23.

2. World Health Organisation. Global tuberculosis control: WHO report 2012. World Health Organisation; 2012.

3. Savine E, Warren RM, van der Spuy GD, Beyers N, Van Helden PD, Locht C, et al. Stability of variable-number tandem repeats of mycobacterial interspersed repetitive units from 12 loci in serial isolates of Mycobacterium tuberculosis. *J Clin Microbiol* 2002;40(12):4561-6.

4. Taype CA, Agapito JC, Accinelli RA, Espinoza JR, Godreuil S, Goodman SJ, et al. Genetic diversity, population structure and drug resistance of Mycobacterium tuberculosis in Peru. *Infect Genet Evol* 2012;12(3):577-85.

5. Becerra MC, Appleton SC, Franke MF, Chalco K, Arteaga F, Bayona J, et al. Tuberculosis burden in households of patients with multidrug-resistant and extensively drug-resistant tuberculosis: a retrospective cohort study. *Lancet* 2011;377(9760):147-52.

6. Mitnick C, Bayona J, Palacios E, Shin S, Furin J, Alcantara F, et al. Community-based therapy for multidrug-resistant tuberculosis in Lima, Peru. *N Engl J Med* 2003;348(2):119-28.

7. Allix-Beguec C, Harmsen D, Weniger T, Supply P, Niemann S. Evaluation and strategy for use of MIRU-VNTRplus, a multifunctional database for online analysis of genotyping data and phylogenetic identification of Mycobacterium tuberculosis complex isolates. *J Clin Microbiol* 2008;46(8):2692-9.

8. Iwamoto T, Grandjean L, Arikawa K, Nakanishi N, Caviedes L, Coronel J, et al. Genetic diversity and transmission characteristics of Beijing family strains of Mycobacterium tuberculosis in Peru. *PLoS One* 2012;7(11):e49651.

9. Blasi F, Reichman LB, Migliori GB. Presenting the European Forum for TB Innovation: innovative thinking in progressing towards TB elimination in Europe. *Eur Respir J* 2012;40(4):806-8.

10. Diel R, Loddenkemper R, Zellweger JP, Sotgiu G, D'Ambrosio L, Centis R, et al. Old ideas to innovate TB control: preventive treatment to achieve elimination. *Eur Respir J* 2013.

Figure 1. The Beijing genotype is enriched with increasing drug resistance in English cases and may be increasingly represented among MDR cases over time.

a) The Beijing genotype is enriched with increasing drug resistance in English (England 1) (p_{trend} <0.005 Beijing versus non-Beijing), but not Peruvian (Peru 1, Peru 2), pulmonary TB cases. b) Lineages of English pulmonary MDR-TB cases (England 1, England 2), presented by year of diagnosis. The proportion of cases associated with the Beijing genotype may be increasing with time in the latter years. CAS- Central Asian, EAI- East-Africa Indian, TB- tuberculosis, *- 24-loci MIRU-VNTR typing was not undertaken between 2008 and 2009.