A Geographically-Restricted but Prevalent
*Mycobacterium tuberculosis* Strain Identified in the West Midlands Region of the UK between 1995 and 2008

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**Abstract**

**Background:** We describe the identification of, and risk factors for, the single most prevalent *Mycobacterium tuberculosis* strain in the West Midlands region of the UK.

**Methodology/Principal Findings:** Prospective 15-locus MIRU-VNTR genotyping of all *M. tuberculosis* isolates in the West Midlands between 2004 and 2008 was undertaken. Two retrospective epidemiological investigations were also undertaken using univariable and multivariable logistic regression analysis. The first study of all TB patients in the West Midlands between 2004 and 2008 identified a single prevalent strain in each of the study years (total 155/3,056 (5%) isolates). This prevalent MIRU-VNTR profile (32333 2432515314 434443183) remained clustered after typing with an additional 9-loci MIRU-VNTR and spoligotyping. The majority of these patients (122/155, 79%) resided in three major cities located within a 40 km radius. From the apparent geographical restriction, we have named this the “Mercian” strain. A multivariate analysis of all TB patients in the West Midlands identified that infection with a Mercian strain was significantly associated with being UK-born (OR = 9.03, 95%CI = 4.56–17.87, p < 0.01), Black Caribbean (OR = 5.68, 95%CI = 2.96–10.91, p < 0.01) resident in Wolverhampton (OR = 9.29, 95%CI = 5.69–15.19, p < 0.01) and negatively associated with age >65 years old (OR = 0.25, 95%CI = 0.09–0.67, p < 0.01). A second more detailed investigation analyzed a cohort of 82 patients resident in Wolverhampton between 2003 and 2006. A significant association with being born in the UK remained after a multivariate analysis (OR = 9.68, 95%CI = 2.00–46.78, p < 0.01) and excess alcohol intake and cannabis use (OR = 6.26, 95%CI = 1.45–27.02, p = .01) were observed as social risk factors for infection.

**Conclusions/Significance:** The continued consistent presence of the Mercian strain suggests ongoing community transmission. Whilst significant associations have been found, there may be other common risk factors yet to be identified. Future investigations should focus on targeting the relevant risk groups and elucidating the biological factors that mediate continued transmission of this strain.

Citation: Evans JT, Serafino Wani RL, Anderson L, Gibson AL, Smith EG, et al. (2011) A Geographically-Restricted but Prevalent *Mycobacterium tuberculosis* Strain Identified in the West Midlands Region of the UK between 1995 and 2008. PLoS ONE 6(3): e17930. doi:10.1371/journal.pone.0017930

Editor: Pere-Joan Cardona, Fundació Institut Germans Trias i Pujol; Universitat Autònoma de Barcelona CibeRES, Spain

Received August 2, 2010; Accepted February 20, 2011; Published March 25, 2011

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Funding: The implementation of prospective DNA fingerprinting was enabled by a grant from the UK Department of Health. The Department of Health had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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**Introduction**

DNA fingerprinting of *Mycobacterium tuberculosis* has a key role in TB control and cluster investigation as the molecular data obtained can be used to direct and focus public health control efforts [1,2]. For example, DNA fingerprinting enhanced the investigation of a large outbreak in North London where many of the epidemiological links would not have been established by routine contact tracing or traditional epidemiological investigations alone [3]. Large-scale studies of *M. tuberculosis* strains have also enabled the assessment of the impact of global strain migration and the transmission dynamics of specific strains on a local or regional level [4–8].

The number of cases of tuberculosis in the UK has consistently increased each year since the late 1980s with 8,655 cases (14.1 cases per 100,000 population) diagnosed in 2008. There were
1,012 clinical cases (18.7 per 100,000) in the West Midlands region of the UK with a 43% increase in case numbers in the region since 2000. Birmingham is the largest city in the West Midlands with a rate of 42.4 cases per 100,000 in 2008. There were 44.3 cases per 100,000 in London in 2008. There is large variation in the incidence of TB across the West Midlands, with rates highest in one urban area of Birmingham (>80 cases per 100,000) and lowest in rural Worcestershire (<4 cases per 100,000 in 2008) [9].

We analyzed all M. tuberculosis isolates in the West Midlands region of the UK from 2004 and 2008 by universal prospective DNA fingerprinting and identified the most prevalent strain. We then examined the geographical distribution and epidemiological characteristics of cases infected with this strain in the West Midlands region, and in the city of Wolverhampton, which was found to have the highest proportion of patients with this strain.

Methods

Study Population

The setting for this study was the West Midlands region of the UK. This region had a total population of 5.4 million in 2008. The city of Birmingham has the largest population in the West Midlands with one million inhabitants [10]. Prospective universal DNA fingerprinting was undertaken between 2004 and 2008 with retrospective genotyping carried out on strains isolated before 2004. Retrospective observational epidemiological investigations were undertaken within one city and on a regional scale.

Case definition

Patients with the MIRU-VNTR profile of the most prevalent M. tuberculosis strain in the West Midlands were included in further epidemiological investigation.

Mycobacterial strains and DNA fingerprinting

The HPA Midlands Regional Centre for Mycobacteriology undertook IS6110 Restriction Fragment Length Polymorphism (RFLP) typing on specific requested M. tuberculosis strains until 2004. From 2004 onwards, all M. tuberculosis isolates received from laboratories in the West and East Midlands at the HPA Midlands Regional Centre for Mycobacteriology at Birmingham Heartlands Hospital identified as members of the M. tuberculosis complex were analysed by MIRU-VNTR (Mycobacterial Interspersed Repetitive Units containing Variable Number Tandem Repeats) typing using 15 loci as previously described [11]. The most recent complete calendar year available for molecular and epidemiological data analysis was 2008. A selection of strains were analysed by MIRU-VNTR between June 2003 and February 2006 to confirm the genetic relatedness within clusters. Strains identified as the Mercian strain MT14323 as a control strain [15].

Confirmation of the most prevalent MIRU-VNTR profile as a single strain by IS6110 RFLP

For M. tuberculosis isolates originally cultured between 1995 and 2003, DNA fingerprinting was undertaken retrospectively on requested clusters with significant epidemiological links. Strains were retrospectively analyzed by MIRU-VNTR and IS6110 RFLP to confirm the genetic relatedness within clusters. IS6110 RFLP interrogates a different and independent genetic sequence than MIRU-VNTR typing. IS6110 RFLP was undertaken in accordance with the international standardized protocol using M. tuberculosis strain MT14323 as a control strain [15].

Assignation of global clade lineage

Spoligotyping was carried out to identify the global strain family that the most prevalent strain is part of. Spoligotyping was performed using the Luminex Multianalyte Profiling System as previously described [16]. Spoligotype families were assigned by comparison to the international SpolDB4 database [17].

West Midlands regional epidemiological analysis

Patients with M. tuberculosis strains identified as the Mercian strain between 2004 and 2008 were compared to all patients with tuberculosis in the West Midlands between 2004 and 2008 in the HPA Enhanced Tuberculosis Surveillance System. The HPA Enhanced Tuberculosis Surveillance System contains molecular, pathological, and treatment data on all notified cases of tuberculosis in England including culture-confirmed cases and clinically diagnosed cases. All patients with strain typing data were selected for comparison. Two levels of patient residence were analyzed: West Midlands Health Protection Unit and Local Authority. Local HPA Health Protection Units work alongside the National Health Service in England providing specialist support in communicable disease and infection control. There are three Health Protection Units in the West Midlands: West Midlands East, West Midlands North, and West Midlands West. The major cities of Birmingham and Coventry are located in West Midlands East, West Midlands North, and Wolverhampton in West Midlands West. More specific analysis of patient residence was undertaken by analyzing patient location within one of 33 Local Authority regions in the West Midlands. Local Authorities are administrative regions that are based on city or county boundaries.

Geographical distribution of the Mercian strain within the West Midlands region

Laboratory records of patients with the most prevalent strain were used to map patient residential location using postcode within the West Midlands.

City specific epidemiological investigation

When it became apparent that there was a cohort of patients in Wolverhampton with an indistinguishable MIRU-VNTR profile, a retrospective review of patient case notes and interview of specialist tuberculosis nurses who were involved with the care of these patients was undertaken for culture-positive patients resident in Wolverhampton diagnosed with the same indistinguishable MIRU-VNTR profile between June 2003 and February 2006 to identify common factors and potential epidemiological links. These patients were compared to culture-positive cases diagnosed with other strains in 2004.

A questionnaire was designed to collect comprehensive epidemiologic information including demographic characteristics, clinical history, predisposing risk factors and evidence of contact
with patients with active disease caused by any strain. Information was also obtained on occupational, social and recreational history, compliance with tuberculosis treatment and change in weight after eight weeks treatment. Chest radiographs of all patients were reviewed for the presence of cavitation.

Statistical analysis

Proportions calculated from epidemiological data obtained from the West Midlands regional and Wolverhampton city datasets were compared using Pearson’s chi-squared test with Fisher’s exact test where necessary. Univariate and multivariate logistic regression modeling was used to test the significance of odds ratios in Stata v10 (Stata Corp, College Station, TX, USA). The multivariable model was assembled by adding covariates individually in decreasing order of significance and the “goodness of fit” of each model was assessed using the likelihood ratio test. All cases with missing values for the variables examined were excluded from the multivariate model with 114 patients infected by the Mercian strain and 1,891 patients in the control group included. Differences in proportions between entries with complete data for each variable and missing data for at least one variable was analysed. A univariate analysis of the epidemiological investigation of patients resident in Wolverhampton was undertaken using EpiData Analysis v2.2 (EpiData Association, Odense, Denmark). The extent of any association was expressed as an odds ratio (OR) with 95% confidence intervals.

Ethics Statement

This report details the current status of the investigation into the most prevalent strain in the West Midlands, which has been undertaken as part of normal public health practice by microbiologists, respiratory physicians, and public health teams. Therefore, specific ethical approval was not required. The Health Protection Agency (HPA) has Patient Information Advisory Group permission under the Health and Social Care Act 2001 to collect and analyse such data for public health purposes.

Results

M. tuberculosis strains

Between 2004 and 2008, 4,830 isolates were typed from 31 referring laboratories in the West and East Midlands. Duplicate isolates (n = 171) were removed so that 4,659 isolates representing the first typed isolate from each patient between 1 January 2004 and 31 December 2008 were selected. From 4,659 isolates typed, 4,659, 2% (117/4,659, 3%) and 42235 2642515333 (88/4,659, 2%).

Case definition

Inclusion of a patient for further epidemiological investigation was based on MIRU-VNTR typing, with a confirmed case defined as a patient with microbiologically confirmed tuberculosis and an isolate that had the 32333 2432515314 MIRU-VNTR profile.

UK distribution of the most prevalent MIRU-VNTR profile in the West Midlands

The HPA UK Mycobacterium tuberculosis Strain Typing Database was interrogated to analyze the national distribution of the most prevalent strain in the West Midlands with a total of 176 isolates identified across the UK between 2004 and 2008. Only 6/162 (4%) of these isolates were identified in patients resident outside of the Midlands. Since this MIRU-VNTR profile appeared to be geographically restricted to the West Midlands in the UK, we have named the profile the “Mercian strain”, after the Anglo-Saxon kingdom of Mercia [18].

Geographical distribution of the Mercian strain within the West Midlands region

Three major cities in the West Midlands were the focus for this strain as 55/156 (35%) isolates originated from Wolverhampton, 41/156 (26%) isolates from Birmingham, and 26/156 (17%) isolates from Coventry. Overall, 122/156 (78%) Mercian isolates were identified in patients resident in one of these three cities which are located within a 40 km radius of each other. The Mercian strain accounted for 21% (53/258) of all M. tuberculosis isolates in Wolverhampton compared to 9% (27/285) of all strains in Coventry and 3% (41/1,243) in Birmingham. Figure 2 shows the geographical mapping of 32333 2432515314 in the West Midlands between 2004 and 2008.

Confirmation of the 32333 2432515314 MIRU-VNTR profile as a distinct strain

Retrospective typing of stored M. tuberculosis isolates resulted in the identification of an additional 51 isolates from six different locations as members of the 32333 2432515314 Mercian strain MIRU-VNTR profile. Upon further investigation by IS6110 RFLP, 50/51 (98%) of these strains were still indistinguishable (Figure 3) with a 7-band RFLP pattern. One isolate from Wolverhampton in 2003 possessed 8 copies of IS6110 but this can still be considered as part of the same strain and the MIRU-VNTR profile did not vary in this single isolate. A selection of 10 strains from five different locations isolated between 2004 and 2008 were analyzed by the optimal 24 MIRU-VNTR loci set and spoligotyping. All 10 strains were indistinguishable at each of the 24 loci (32333 2432515314 434443183) and possessed an indistinguishable spoligotype (octal type 76776777760771) which is shared type (ST) 490 and is a member of the Clade X1 global clade. Investigation of the global SpolDB4 database revealed that only 3 strains with this shared type have been identified. The 3 other strains that are members of ST490 were identified as single strains in London, New York and Washington, DC.

West Midlands regional epidemiological analysis

A total of 124/156 (79%) tuberculosis patients with the Mercian strain were successfully matched to notification data in the HPA Enhanced Tuberculosis Surveillance system. There were 2,066 tuberculosis patients with other strain types notified in the West Midlands between 2004 and 2008. Patient characteristics identified as risk factors significant in a univariate analysis (Table 1 and Table S1 for all epidemiological variables) were residence in the West Midlands West Health Protection Unit Area and then specifically residence in Wolverhampton, UK-born, and Black Caribbean or White ethnic group. Significant negative associations were identified with age not greater than 65 years old, the Black African ethnic group or extra-pulmonary disease. No significant association with resistance to any of the 1st line
tuberculosis drugs \( (p = .79) \) or multi-drug resistance \( (p = .92) \) was identified. The significant variables were then included in a multi-variate logistic regression which identified that being UK-born, Black Caribbean ethnic group, \( > 65 \) years old, and resident in Wolverhampton, were significantly associated with the Mercian strain (Table 1). Age \( > 65 \) years old was a significantly negative association with the Mercian strain. Therefore, age \( < 65 \) years old is positively associated with the Mercian strain. There were no statistically significant differences between patients with complete data for each variable compared to those with missing data in both this analysis and the following city-wide epidemiological investigation.

City-wide epidemiological investigation in Wolverhampton

The Mercian strain in Wolverhampton was significantly associated with white UK-born patients who presented with cavitations on chest X-ray and produced smear positive specimens (Table 2 and Table S2 for all epidemiological variables). Patients infected with the Mercian strain continued to experience weight loss at 8 weeks after starting anti-tubercular chemotherapy. This result was statistically significant \( (p < 0.05) \). However, there was no significant difference between treatment completion rates after 12 months.

Examination of the epidemiological factors revealed that cases with the Mercian strain were more likely to have a previous history of TB \( (9/35, 26\%) \), and would have had significant previous contact with a case of TB \( (24/35, 69\%) \), and in particular patients with the Mercian strain \( (13/35, 37\%) \) (Table 2). Significant social factors detected were evidence of excess alcohol intake and cannabis use.

Discussion

We describe here the identification of the most prevalent \textit{M. tuberculosis} strain in the West Midlands, which we have termed the Mercian strain. Concordant MIRU-VNTR and RFLP data from six different geographical locations across the West Midlands indicated that this strain is present in 3 major cities. Regional, national, and global genotyping databases provided evidence that this strain was restricted to the West Midlands region in England. Regional data showed that this strain primarily infected UK-born, Black Caribbean patients less than 65 years old.

The regional and Wolverhampton epidemiological investigations presented in this report identified significant associations for the Mercian strain. However, they do not provide a full explanation of why the Mercian strain is more prevalent compared to other strains in the West Midlands. Drug and alcohol use were identified as significant social factors in Wolverhampton. Alcohol and drug use have been identified as significant associations in previously reported tuberculosis outbreaks particularly in low-incidence countries [19–22]. The cumulative number of cases and continuing presence of the Mercian strain does not follow a typical point-source outbreak pattern. The significant association with younger age suggests that cases caused by the Mercian strain have arisen as a result of recent transmission and not re-activation in older patients. A possible transmission scenario is that after the initial emergence of the Mercian strain there have been several
independent clusters of transmission each with their own common social link. This has resulted in a large, complex social network where transmission persists and the complete transmission scenario is yet to be fully elucidated.

Both epidemiological investigations presented in this report were retrospective and did not involve direct patient interviews. The Mercian strain continues to be identified in the West Midlands which means that enhanced epidemiological knowledge could be obtained by prospectively investigating social links as each new patient is diagnosed. Investigation of potential factors which may cause a delay in diagnosis should be investigated as well. The data presented by us identified the infected patient population and also important common social factors. The exact interaction of patient population and social factors should be investigated further to identify and fully understand any confounding factors.

It must be noted that the Wolverhampton epidemiological investigation applied a detailed questionnaire that was only used in this location. Of the three major cities in the West Midlands, Wolverhampton had the highest proportion of the Mercian strain (21%). Patients with the Mercian strain in Birmingham and Coventry might differ in their use of drugs and alcohol. The results from the Wolverhampton and region-wide analysis do not concur exactly as different ethnic population groups were identified as at highest risk: the White population in Wolverhampton but the Black Caribbean group across the West Midlands.

Detection of this strain was only possible with the commencement of universal prospective typing of all *M. tuberculosis* isolates in the West and East Midlands. Only with universal prospective DNA fingerprinting was the full extent of the Mercian strain in the West Midlands fully characterized. Since the Mercian strain is not a drug-resistant strain without associated phenotypic
properties that could differentiate it from other *M. tuberculosis* complex strains, it would only have been possible to detect this strain by universal prospective typing. The patient population in which the Mercian strain has been identified is different to the UK-wide situation for TB as the majority of patients diagnosed each year in the UK are not born in the UK and originate from the Indian Sub-Continent [9].

The 156 individual patients detected between 2004 and 2008, make the Mercian strain one of the largest known community-based clusters in the world. Previous major prevalent strains have been identified in New York [23,24], Rotterdam [25], North London [3], and Rio de Janeiro [26].

The most prevalent strain detected in the UK was an isoniazid resistant strain in North London that was previously reported in 70 patients [3], with a current total of over 300 cases caused by this strain (Ibrahim Abubakar, Consultant Epidemiologist & TB Section Head, Respiratory Diseases Department - Tuberculosis Section, Health Protection Agency, personal communication). Isoniazid resistance acted as a very useful marker for detection of the strain. It was noted that without the drug resistance marker only prospective typing of all isolates would have detected this large, complex outbreak. This strain was predominantly found in young White or Black Caribbean UK-born adults with drug misuse as a common epidemiological factor [3]. It is possible that patients in this population group take longer to present clinically as TB may not be suspected when initial symptoms develop or they might not seek medical help soon after onset. Both factors aid strain transmission and disease progression.

A strain was identified in 93/314 (30%) patients in Rio de Janeiro that uniquely lacked a major region of genomic DNA (>26.3 kb) which contained 10 genes including two potentially immunogenic PPE genes [26,27]. The RD® strain was associated with a higher frequency of cavitary pulmonary disease [28]. The major deletion identified in the RD® strain has been hypothesized as having a major impact on the virulence properties of the RD® strain. As the genomic content of the Mercian strain has not been characterized, further work should determine whether such a deletion or other similar major genomic variation has altered the virulence of this strain leading to multiple transmission events in and between three cities in the West Midlands.

Retrospective epidemiological studies have identified the earliest isolate of the Mercian strain from 1995 in an archive strain collection. This isolate was part of a cluster of 11 isoniazid resistant strains identified between 1995 and 2000 which was reported previously before the full regional extent of the Mercian strain was known [2]. We have typed very few archived *M. tuberculosis* strains from 1995 so the full extent of drug sensitive and drug resistant Mercian strains 15 years ago has not yet been assessed. The cluster of isoniazid resistant Mercian strains was present in one specific location. From 1995–2000, there was no other investigated instance of increased isoniazid resistance in the rest of the West Midlands caused by the Mercian strain.

As the Mercian strain has been present since prospective DNA fingerprinting was commenced in 2004 with a median of 30 isolates per year (range 27–37) and has represented a consistent proportion of strains (Figure 1), it is likely that the Mercian strain first emerged in the West Midlands well before 2004.
Table 1. Univariate and multi-variate analysis of sociodemographic, clinical, and bacteriological data for patients with the Mercian strain (n = 124) and all other patients with strain typing data (n = 2,066) in the West Midlands between 2004 and 2008.

| Variable                                      | No. patients | Unadjusted | Adjusted |
|-----------------------------------------------|--------------|------------|----------|
|                                               | Mercian (n = 124) | WT (n = 2,066) | Odds Ratio | 95% CI | P | Odds Ratio | 95% CI | p |
| Gender                                        |              |            |          |        |    |           |        |    |
| Male                                          | 77           | 1,118      | 1.38     | 0.95–2.01 | 0.09 | 1.03      | 0.65–1.62 | 0.91 |
| Age group (years)                             |              |            |          |        |    |           |        |    |
| 0–14                                          | 4            | 45         | 1.27     | 0.45–3.59 | 0.69 | 0.35      | 0.07–1.63 | 0.18 |
| 15–44                                         | 92           | 1310       | 1.00     | 1.00     |      | Reference |        |    |
| 45–64                                         | 23           | 379        | 0.86     | 0.54–1.38 | 0.543| 0.77      | 0.42–1.41 | 0.40 |
| >65                                           | 5            | 330        | 0.22     | 0.09–0.53 | <0.01*| 0.25      | 0.09–0.67 | <0.01*|
| HPU location in the West Midlands             |              |            |          |        |    |           |        |    |
| East                                          | 47           | 1,126      | 1.00     | 1.00     |      | Reference |        |    |
| North                                         | 10           | 206        | 1.16     | 0.58–2.34 | 0.67 |          |        |    |
| West                                          | 67           | 727        | 2.21     | 1.50–3.24 | <0.01*|          |        |    |
| Local Authority                               |              |            |          |        |    |           |        |    |
| Wolverhampton                                 | 51           | 169        | 7.83     | 5.30–11.58 | <0.01*| 9.29      | 5.69–15.19 | <0.01*|
| Place of birth                                |              |            |          |        |    |           |        |    |
| UK-born                                       | 100          | 546        | 18.03    | 10.22–31.81 | <0.01*| 9.03      | 4.56–17.87 | <0.01*|
| Ethnic group                                  |              |            |          |        |    |           |        |    |
| Black Caribbean                               | 32           | 70         | 14.84    | 8.55–25.77 | <0.01*| 5.68      | 2.96–10.91 | <0.01*|
| Black African                                 | 1            | 362        | 0.09     | 0.01–0.68 | 0.02 | 0.19      | 0.02–1.51 | 0.12 |
| Indian Sub-Continent                          | 33           | 1,102      | 1.00     | 1.00     |      | Reference |        |    |
| Other                                         | 6            | 105        | 1.57     | 0.60–4.10 | 0.36 | 1.11      | 0.37–3.37 | 0.85 |
| White                                         | 49           | 366        | 4.47     | 2.79–7.15 | <0.01*| 1.75      | 0.95–3.22 | 0.07 |
| Site of disease                               |              |            |          |        |    |           |        |    |
| Pulmonary sputum smear positive               | 59           | 655        | 1.00     | 1.00     |      | Reference |        |    |
| Pulmonary sputum smear other                  | 47           | 711        | 0.73     | 0.49–1.09 | 0.13 | 1.06      | 0.64–1.74 | 0.83 |
| Extra pulmonary                               | 18           | 685        | 0.29     | 0.17–0.50 | <0.01*| 0.62      | 0.33–1.18 | 0.15 |
| Clinical history of TB                        |              |            |          |        |    |           |        |    |
| Previous diagnosis of TB                      | 10           | 85         | 0.60     | 0.30–1.20 | 0.15 |          |        |    |
| Treatment                                     |              |            |          |        |    |           |        |    |
| Patient admitted as in-patient                | 25           | 329        | 1.04     | 0.64–1.65 | 0.89 |          |        |    |
| Treatment outcome at 12 months                |              |            |          |        |    |           |        |    |
| Treatment completed                           | 71           | 1,157      | 1.00     | 1.00     |      | Reference |        |    |
| Died                                          | 3            | 108        | 0.45     | 0.14–1.46 | 0.19 |          |        |    |
| Lost to follow up                             | 4            | 72         | 0.91     | 0.32–2.55 | 0.85 |          |        |    |
| Still on treatment                            | 2            | 32         | 1.02     | 0.24–4.34 | 0.98 |          |        |    |
| Treatment stopped                             | 1            | 6          | 2.72     | 0.32–22.87 | 0.36 |          |        |    |
| Transferred out                               | 0            | 18         | -        | -        | -    |          |        |    |
| Not completed unknown                         | 2            | 21         | 1.55     | 0.36–6.75 | 0.56 |          |        |    |
| Unknown                                       | 2            | 13         | 2.51     | 0.56–11.32 | 0.23 |          |        |    |
| Treatment outcome at 12 months                |              |            |          |        |    |           |        |    |
| Successful                                    | 73           | 1,189      | 0.89     | 0.47–1.66 | 0.71 |          |        |    |
| Drug Sensitivity Testing                      |              |            |          |        |    |           |        |    |
| Resistance to any 1st line drug               | 5            | 93         | 0.89     | 0.35–2.22 | 0.79 |          |        |    |
| MDR                                           | 1            | 15         | 1.10     | 0.14–8.43 | 0.92 |          |        |    |

*P-values were considered as statistically significant if \( \leq 0.05 \). Significant unadjusted values were included in the multi-variate model.

The wild-type (WT) consisted of tuberculosis patients infected with *M. tuberculosis* strains other than the Mercian strain.

doi:10.1371/journal.pone.0017930.t001
Universal prospective DNA fingerprinting is an essential part of many countries TB control programs as it has been used to estimate transmission in specific population groups. The Netherlands have undertaken universal prospective DNA fingerprinting of all *M. tuberculosis* isolates since 1993 [29]. This enabled the identification of transmission after migration in patients recently arrived in the Netherlands as infections in 30% to 40% of Turkish, Moroccan, and Somali patients could be attributed to recent transmission [29].

Secondly, it has also been shown in the Netherlands that combining data obtained from nationwide tuberculosis contact investigation with DNA fingerprinting can be used to identify transmission routes. Table 2 shows the results of a city-wide investigation of patients with and without the Mercian strain in Wolverhampton, UK between 2003 and 2006.

Table 2. Epidemiological data obtained from a city-wide investigation of patients with and without the Mercian strain in Wolverhampton, UK between 2003 and 2006.

| Variable                      | Mercian (n = 35) | WT (n = 47) | Unadjusted   | Adjusted     |
|-------------------------------|-----------------|-------------|--------------|--------------|
| Unadjusted Odds Ratio         | 95% CI          | p           | Odds Ratio   | 95% CI       | p           |
| Patient gender                |                 |             |              |              |
| Female                        | 18              | 20          | 1.42         | 0.54–3.77    | 0.43        |
| Age group (years)             |                 |             |              |              |
| 0–14                          | 1               | 1           | 1.15         | 0.01–9.299   | 1.00        |
| 15–44                         | 27              | 31          | 1.00         | 1.00         | Reference   |
| 45–64                         | 4               | 10          | 0.46         | 0.10–1.85    | 0.22        |
| >65                           | 3               | 5           | 0.69         | 0.10–3.95    | 0.72        |
| Ethnic group                  |                 |             |              |              |
| Indian Sub-Continent          | 11              | 23          | 1.00         | 1.00         | Reference   |
| Black African                 | 0               | 6           | -            | -            |             |
| Black Caribbean               | 9               | 6           | 3.06         | 0.75–13.46   | 0.07        |
| Other                         | 1               | 5           | 0.44         | 0.01–4.71    | 0.65        |
| White                         | 14              | 7           | 4.06         | 1.15–15.77   | 0.01*       |
| Country of birth              |                 |             |              |              |
| UK-born                       | 32              | 12          | 29.42        | 7.32–177.18  | <0.01*      |
| Previous contact with TB case | 24              | 11          | 6.94         | 2.42–21.50   | <0.01*      |
| Previous contact with Mercian strain | 13          | 0           | 15.65        | 4.76–51.48   | <0.01*      |
| Previous history of TB        | 9               | 3           | 4.97         | 1.11–31.13   | 0.01*       |
| Malignancy                    | 1               | 0           | 10.41        | 0.20–547.59  | 0.43*       |
| Diabetes                      | 2               | 7           | 0.35         | 0.03–2.01    | 0.29        |
| Excess alcohol use            | 16              | 4           | 8.78         | 2.42–41.01   | <0.01*      |
| Cigarette smoking             | 6               | 3           | 2.99         | 0.58–19.96   | 0.12        |
| Cannabis use                  | 11              | 2           | 10.02        | 1.96–100.33  | <0.01*      |
| Unemployed                    | 21              | 23          | 1.56         | 0.59–4.18    | 0.32        |
| Cavitory disease              | 22              | 12          | 4.83         | 1.74–14.24   | <0.01*      |
| Pulmonary disease             | 31              | 34          | 2.93         | 0.79–13.64   | 0.07        |
| Sputum specimen               | 25              | 24          | 2.37         | 0.87–6.84    | 0.06        |
| Positive microscopy           | 22              | 17          | 2.94         | 1.10–8.19    | 0.02*       |
| Therapy non-adherence         | 7               | 3           | 3.61         | 0.75–23.42   | 0.06        |
| Weight loss after initiation of treatment | 8            | 0           | 12.99        | 3.00–56.28   | <0.01*      |
| Completed treatment within 12 months | 26           | 36          | 0.64         | 0.17–2.29    | 0.43        |
| Resistance to any 1st line drug | 0              | 1           | -            | -            |             |
| MDR                            | 0               | 0           | -            | -            |             |

*P-values were considered as statistically significant if ≤0.05.

**OR for excess alcohol use and cannabis use combined.

doi:10.1371/journal.pone.0017930.t002

Universal prospective DNA fingerprinting is an essential part of many countries TB control programs as it has been used to estimate transmission in specific populations groups. The Netherlands have undertaken universal prospective DNA fingerprinting of all *M. tuberculosis* isolates since 1993 [29]. This enabled the identification of transmission after migration in patients recently arrived in the Netherlands as infections in 30% to 40% of Turkish, Moroccan, and Somali patients could be attributed to recent transmission [29].

Secondly, it has also been shown in the Netherlands that combining data obtained from nationwide tuberculosis contact investigation with DNA fingerprinting can be used to identify transmission routes.
investigation and DNA fingerprinting surveillance greatly increased the number of defined epidemiological links. In 2,206 clustered cases, DNA fingerprinting increased the number of epidemiologic links from 462 before DNA fingerprinting data was known to 1,062 epidemiologically established links after cluster investigation involving a combination of molecular and epidemiological data. DNA fingerprinting did not increase the number of patients identified as contacts but cluster monitoring did enable the identification of transmission events not detected by contact investigations, the development and evaluation of focused interventions and evaluation of regional tuberculosis eradication programs [30]. A study in Maryland showed that cluster investigation including DNA fingerprinting analysis identified 43/113 (38%) of all detected patient links. [31]

A more recent study showed that DNA fingerprinting data could be used to prospectively identify rapidly expanding clusters before expansion actually occurred based on the properties of the first two patients in a cluster. If the first two patients in a cluster were identified within 3 months of each other, one or both were <35 years old, and both patients resided in an urban area and originated from Sub-Saharan Africa, there was a more than 5 times increased probability that this strain in an initial cluster of two patients in an epidemiologically defined cluster would be identified in 5 or more patients within 2 years [32].

We describe here the identification of the most prevalent M. tuberculosis strain in the West Midlands region of the UK, with 156 isolates in a 5 year period between 2004 and 2008. The Mercian strain has been significantly associated with UK-born patients, appears to be geographically restricted to the West Midlands region in the UK with evidence of ongoing transmission.

**Supporting Information**

**Table S1** Univariate and multi-variate analysis of sociodemographic, clinical, and bacteriological data for patients with the Mercian strain (n = 124) and all other patients with strain typing data (n = 2,066) in the West Midlands from 2004–2008.

**Table S2** Epidemiological data obtained from a city-wide investigation of patients with and without the Mercian strain in Wolverhampton, UK.

**Acknowledgments**

The authors would like to thank all microbiology laboratories in the Midlands that refer specimens and isolates to the Midlands Regional Centre for Mycobacteriology, local tuberculosis clinical services and health protection units, all centres that contribute strain typing data to the HPA UK M. tuberculosis Strain Typing database and all staff at the Midlands Regional Centre for Mycobacteriology for undertaking isolation, identification, and typing of isolates. The authors would like to specifically acknowledge: Helen Bagnall and Jianxia Sun (HPA West Midlands Regional Surveillance Unit, Birmingham, UK) for providing the geographical mapping and treatment outcome data for the Wolverhampton database; Richard Myers and Jonathan Green (Bioinformatics Unit, HPA Centre for Infections, London, UK) for advice with regards to querying the HPA national typing database and providing geographical matching to NHS geographical boundaries; Evarn Arnold (HPA Centre for Infections, London, UK) for statistical advice; Beck Taylor (Department of Public Health, Epidemiology and Biostatistics, University of Birmingham, Birmingham, UK); Ashin Banerjee (NHS Worcestershire, Worcester, UK); Huda Mohamed (HPA West Midlands East Health Protection Unit, Solihull, UK), John Innes (Birmingham Chest Clinic, Birmingham, UK) and Naveed Syed (HPA West Midlands West Health Protection Unit, Kidderminster, UK) for various investigations into the Mercian strain.

**Author Contributions**

Conceived and designed the experiments: JTE RLSW LA ALG EGS AW IA JS PMH. Performed the experiments: JTE RLSW LA ALG SG. Analyzed the data: JTE RLSW LA ALG SG. Contributed reagents/materials/analysis tools: RLSW LA BO IA JSM SG HJ. Wrote the paper: JTE LA EG AW IA PS PMH.

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