Published in final edited form as:

Int J Tuberc Lung Dis. 2012 October ; 16(10): 1377–1382. doi:10.5588/ijtld.11.0709.

Tuberculosis caused by RD^{Rio} *Mycobacterium tuberculosis* is not associated with differential clinical features

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Abstract

BACKGROUND—We recently described the *Mycobacterium tuberculosis* RD^{Rio} genotype, a clonally derived sublineage within the Latin American–Mediterranean (LAM) family. Genetic diversity of *M. tuberculosis* likely affects the clinical aspects of tuberculosis (TB). Prospective studies that address this issue are scarce and remain controversial.

OBJECTIVE—To determine the association of differential clinical features of pulmonary TB with the RD^{Rio} *M. tuberculosis* etiology.

METHODS—Culture-proven pulmonary TB patients ($n = 272$) were clinically evaluated, including history, physical examination, chest X-ray and anti-human immunodeficiency virus serology. Isolates were classified as RD^{Rio} or non-RD^{Rio} *M. tuberculosis* by multiplex polymerase chain reaction and further spoligotyped. Clinical and *M. tuberculosis* genotype data were analyzed.

RESULTS—RD^{Rio} *M. tuberculosis* caused disease in 26.5% (72/270) of all TB cases. The LAM genotype, of which RD^{Rio} strains are members, was responsible for 46.0% of the TB cases. Demographic data, major signs and symptoms, radiographic presentation, microbiological features and clinical outcomes were not significantly different among patients with TB caused by RD^{Rio} and non-RD^{Rio} strains.

CONCLUSIONS—Disease caused by *M. tuberculosis* RD^{Rio} strains was not clinically distinctive or more severe than disease caused by non-RD^{Rio} strains in this series of TB patients. Larger prospective studies specifically designed to disclose differential clinical characteristics of TB caused by specific *M. tuberculosis* lineages are needed.

Keywords

Mycobacterium tuberculosis; lineage; RD^{Rio}; epidemiology; clinical features

A leading cause of death worldwide, tuberculosis (TB) is primarily a disease of the lungs, caused by very closely related mycobacteria belonging to the *Mycobacterium tuberculosis* complex (MTC). Genotyping of MTC isolates is used to identify mycobacteria at species level and distinguish MTC into distinct lineages and/or sublineages that are a powerful tool for TB epidemiological control, phylogenetic exploration and investigation of host-pathogen interactions.

The highly structured population genetics of MTC is defined by six major phylogeographic lineages: *M. africanum* (West-African lineages 1 and 2), *M. bovis* and four *M. tuberculosis* lineages: Indo-Oceanic (formerly referred as ancestral), Euro-American, East Asian (mostly Beijing) and East-African Indian (EAI).¹ These major lineages can be definitively characterized using long sequencing polymorphisms (LSPs).² More often, however, for the purposes of TB surveillance, the less precise spoligotyping and mycobacterial interspersed repetitive units-variable number tandem repeats (MIRU-VNTR) are used.

The Latin American–Mediterranean (LAM) genotype is a member of the Euro-American lineage of *M. tuberculosis* and is the dominant genotype responsible for approximately half of Brazilian TB cases.^{3–6} In previous studies, we described that the clonally derived RD^{Rio} strains of *M. tuberculosis* are defined by a ~26.3 kb deletion resulting in the loss or modification of 10 genes, including two preproenkephalin genes known to be recognized by host immunity.⁷ In prior publications from our group, insertion sequence (IS) *6110* restriction fragment length polymorphism and MIRU-VNTR typing showed that the RD^{Rio} sublineage likely occurred at a more distant evolutionary time, as both methods indicated considerable divergence, while it remains in the Euro-American *M. tuberculosis* lineage as well as belonging to the single nucleotide polymorphism Cluster VI grouping.^{6–8}

RD^{Rio} *M. tuberculosis* strains, present in at least 18 other countries of Europe, Africa and the Americas, are all members of the LAM genotype and a sublineage of the EuroAmerican lineage.^{6,7,9} RD^{Rio} *M. tuberculosis* is the most prevalent cause of TB in Rio de Janeiro and has also been reported in large numbers throughout other Brazilian regions.^{4,7,10} Results from recent studies suggest that *M. tuberculosis* lineages are adapted to particular human populations, and that *M. tuberculosis* is older and more genetically diverse than previously assumed.^{1,11} It is likely that such genetic diversity could result in lineage-specific biological characteristics, ultimately influencing both the clinical and epidemiological aspects of TB. However, information on the nature of coupling between *M. tuberculosis* lineages and the development of distinctive clinical manifestations of TB is scarce, and this issue remains somewhat enigmatic. In the present prospective study, we sought to determine whether there were distinct demographic, clinical, radiologic and laboratory features associated with pulmonary TB caused by RD^{Rio} *M. tuberculosis* strains.

MATERIALS AND METHODS

Study setting and design

A total of 278 consenting adult patients with culture-proven pulmonary TB, diagnosed at the Federal University of Rio de Janeiro (FURJ) and two community health centers were enrolled in the study. A thorough clinical evaluation, including history, physical examination, chest X-ray (CXR), anti-human immunodeficiency virus (HIV) serology, tuberculin skin test (TST) and patient follow-up for at least 1 year after completion of treatment were performed as part of routine clinical care. CXRs, performed before starting treatment, were independently evaluated and scored in a standardized form by two experienced pulmonologists blinded to the RD^{Rio} genotype. In cases of disagreement, discussion among readers led to a decision by consensus on the final scoring. Of 217 films, 11 were excluded due to poor quality, and CXRs were not available for analysis in 57 of the 272 TB cases.

The study protocol was approved by the Institutional Review Board of the FURJ University Hospital. Signed informed consent was obtained from all patients.

Phenotypic, biochemical and genetic characterization of *M. tuberculosis* isolates

Mycobacterial (Löwenstein-Jensen) culture and phenotypical and biochemical characterization of isolates from all patients were performed according to standard methods.¹² Drug susceptibility testing (DST) against anti-tuberculosis drugs was performed using the proportion method.¹³ The average time until *M. tuberculosis* growth detection in cultures and bacillary load determination using quantitative acid-fast bacilli (AFB) smears and colony-forming unit assays were recorded on admission and at 8 weeks of treatment. A multiplex polymerase chain reaction (PCR) assay of all 278 *M. tuberculosis* culture thermolysates was performed on these isolates to differentiate RD^{Rio} from other lineage strains, as described by Gibson et al.⁹ For further genotyping, DNA from *M. tuberculosis* isolates was extracted and spoligotyping performed using PCR and hybridization conditions on a commercially available membrane (Isogen Bioscience BV, Maarssen, The Netherlands), as previously described.¹⁴ Spoligotype patterns were recorded both in a 43-digit binary format representing the 43 spacers and as an octal code.¹⁵ *M. tuberculosis* patterns were introduced into a Microsoft Office Excel datasheet (Microsoft, Redwoods, WA, USA) for cluster recognition and classification and then compared with the SpolDB4 database of the Pasteur Institute of Guadeloupe (<http://www.pasteur-guadeloupe.fr:8081/SITVITDemo>) for type classification.

Data analysis

Data were summarized by median and standard deviation for continuous variables and by frequency and proportion for categorical variables. Categorical variables were compared for RD^{Rio} and non-RD^{Rio} sublineages using the χ^2 and Fisher's exact tests. The Mann-Whitney test was applied for continuous variables. Odds ratios (ORs) and their 95% confidence intervals and *P* values were estimated. Statistical analysis was performed using GraphPad Prism, version 4.02 (GraphPad Software, La Jolla, CA, USA). *P* < 0.05 was considered significant.

RESULTS

Pulmonary *M. tuberculosis* isolates were obtained from sputum (72.1%), induced sputum (25.4%) and bronchoalveolar lavage fluid (2.5%). All examined isolates, corresponding to the 278 enrolled culture-positive pulmonary TB patients, were characterized as *M. tuberculosis* using biochemical methods, further submitted to multiplex PCR and grouped as

RD^{Rio} or non-RD^{Rio} sublineage. Six patients with pulmonary specimens presenting two different strains and/or an indeterminate RD^{Rio}/non-RD^{Rio} genotype were excluded from the final analysis. The RD^{Rio} *M. tuberculosis* was the cause of disease in 26.5% of the patients in this series. Spoligopatterns were generated for 189/272 isolates (70.2%); all presented genotypes characteristic of *M. tuberculosis*. Altogether, the LAM family was responsible for 46.0% of the TB cases in this series. Based on spoligotype analysis, all RD^{Rio} strains belong to LAM 9, LAM 1 and/or LAM 2 strains, whereas the LAM 3 genotype was found exclusively among non-RD^{Rio} strains. In addition to LAM, the T, Haarlem, U, X, Beijing and Beijing-like families were observed among non-RD^{Rio} strains (Table 1). No orphan patterns were observed.

Overall, clinical data showed that most patients were AFB smear-positive (79.4%) and had advanced disease on CXR, with infiltrates involving more than one lobe (66.5%) and/or cavitation(s) (53.8%). The rate of TB-HIV co-infection was 8.5%. The characteristics of patients infected with RD^{Rio} *M. tuberculosis* strains ($n = 72$) were compared with those infected with non-RD^{Rio} strains ($n = 200$). Socio-demographic and clinico-radiological data are summarized in Table 2. The majority of our patients were male (68.0%). Age, sex, HIV status, CXR appearance (including presence of cavitations and disease extension) and frequencies of smoking, bacille Calmette-Guérin scar, positive TST, response to treatment, previous anti-tuberculosis treatment and/or recurrence after completion of a previous standard TB treatment were not significantly different among patients with TB caused by RD^{Rio} and those with non-RD^{Rio} strains. On entering the study, patients did not differ significantly as regards presence of cough, chest pain, expectoration, hemoptysis, weight loss, fever (Table 2), or duration of symptoms (data not shown). Interestingly, anorexia was significantly more frequent among RD^{Rio} TB patients; however, the nutritional status in both groups of patients was similar, as judged by body mass index (BMI; 19.9 vs. 21.3 kg/m²) and weight gain during the first 2 months of treatment. Microbiological features of RD^{Rio} and non-RD^{Rio} pulmonary TB patients are presented in Table 3. The average time until *M. tuberculosis* growth detection in cultures, bacillary load and frequencies of positive AFB smears, single-drug resistance and multidrug-resistant TB (MDR-TB) were very similar among both groups of TB patients.

DISCUSSION

M. tuberculosis has evolved in large, divergent clonal lineages that are geographically constrained. Importantly, specific ethnic groups seem to have distinct susceptibilities to particular *M. tuberculosis* strains.^{1,6,11,15} Thus, along with interactions of social, environmental and host genetic backgrounds, the *M. tuberculosis* genetic diversity likely affects both the clinical and epidemiological aspects of TB in a lineage-specific way. The *M. tuberculosis* Euro-American superlineage, in which the LAM genotype family is strongly predominant, is the leading etiology of TB in Brazil. In a recent study, it was identified in respectively 96.4% and 66.8% of the analyzed isolates.⁴ We recently described that the RD^{Rio} genotype, defined by an ~26.3 kb deletion, is a clonally derived sublineage of the Euro-American superlineage and a member of the LAM family.^{6,7,9} The *M. tuberculosis* RD^{Rio} sublineage is the most prevalent cause of TB in Rio de Janeiro and is also present in other Brazilian and world regions.^{6,8,9,16,17} In accordance with our previous results,^{6,7,9} the present work confirmed RD^{Rio} exclusively as a LAM sublineage. Indeed, RD^{Rio} comprised more than half of the LAM strains (57.4%). Most of the strains with an LAM9 spoligopattern were of the RD^{Rio} sublineage (86.5%), and all LAM1 and LAM2 spoligotypes were RD^{Rio} sublineage, while the LAM3 spoligotype was exclusively of the non-RD^{Rio} genotype.

In the present study, pulmonary TB caused by the *M. tuberculosis* RD^{Rio} sublineage in Rio de Janeiro was not clinically distinctive or more severe than TB caused by non-RD^{Rio} *M. tuberculosis* strains with respect to patient demographic data, major signs and symptoms, radiographic presentation, microbiological features, frequency of resistant strains and treatment outcome. Although lineage-specific differences in host ethnic preference and TB epidemiology are beginning to emerge,^{1,6,11,18–21} studies correlating *M. tuberculosis*-specific lineage to differential clinical patterns of TB are scarce and often present conflicting results. Differential radiographic patterns have been reported by some,^{10,22,23} but not others.^{6,17,18} It was observed that Indonesian TB patients infected with Beijing strains more often had a febrile response to treatment,¹⁸ while the contrary was reported in Russia.²³ In Taiwan, no difference in treatment outcomes were found between W-Beijing and non-W-Beijing groups.¹⁹ In fact, with the exception of a few studies that have looked at the *M. tuberculosis* lineage etiology of extra-pulmonary TB,^{20,22} frequencies of MDR-TB^{21,23,24} and progression to severe disease,^{11,16,22} most data have shown preponderantly marginal effects on few clinical parameters.^{7,10,17–19} In addition, the drug resistance observed in studies looking at MDR-TB and lineages may be acquired and not clonally derived.²⁵

Overall, our results are in agreement with previously published works that failed to disclose a clear-cut and/or specific association between *M. tuberculosis* lineage and major TB clinical features.^{7,10,17–19,26,27} Nevertheless, for several reasons, these results do not convincingly preclude the importance of *M. tuberculosis* lineage specificity in TB pathogenesis: 1) there is evidence from in vitro^{28,29} and in vivo^{30–32} research suggesting that genotype plays a role in pathogenicity; 2) epidemiological studies indicate that some *M. tuberculosis* genotypes, including RD^{Rio}, may have different transmission rates favoring unequal distribution of *M. tuberculosis*;^{6,11,18,22,24} 3) we do not know that all strains belonging to a particular lineage will share the same pathogenicity factors; 4) genetically distinct specific *M. tuberculosis* lineage/sublineage strains may be circulating; 5) some associations seem to be true to some patient populations but not others;^{10,18,23} 6) socio-economic and cultural barriers to effective medical care may increase the time elapsed before diagnosis, masking major clinical differences; 7) often only a small number of patients are analyzed thoroughly and in depth; 8) longitudinal specific study design is rarely used; and 9) most studies lack data on a key variable: TB recurrence after completion of adequate anti-tuberculosis treatment in a context of high treatment completion rates.

In the present study, response to current treatment, default rate, previous anti-tuberculosis treatment and/or recurrence after completion of a previous standard anti-tuberculosis regimen were not significantly different among patients with TB caused by RD^{Rio} and those with non-RD^{Rio} *M. tuberculosis* strains. In addition, microbiologic parameters, such as the average time until detection of *M. tuberculosis* growth in cultures, bacillary load in cultures and frequencies of positive AFB smears before starting treatment and after 2 months of treatment, and frequencies of single or multiple drug resistance, were similar. Although criticized, 2-month sputum culture is the most widely used putative surrogate for prediction of treatment outcome and relapse.³³ The similar bacillary load of sputum cultures of RD^{Rio} and non-RD^{Rio} TB patients at 2 months of treatment suggests that, considering this somewhat limited criterion, a major, clinically relevant difference in virulence between RD^{Rio} and non-RD^{Rio} strains is unlikely. This is in agreement with in vitro fitness analysis comparing these strains.²⁸

The frequencies of medical conditions that have been associated with increased TB risk, namely HIV infection, diabetes mellitus and tobacco smoking, were similar in both groups, suggesting that RD^{Rio} strains were not more infective and/or virulent among patients presenting one of these causes of immune dysfunction. Among all clinical parameters analyzed, only anorexia showed a significantly different frequency among the two groups,

occurring much more frequently among non-RD^{Rio} TB patients. However, the nutritional status in both groups was similar, as based on BMI, and the rate of weight gain during the first 2 months of treatment was also comparable.

The lack of sublineage association with most major clinical parameters (MDR-TB, HIV co-infection, clear clinical and microbiological differences in response to treatment), observed in the present work, are in agreement with most of our earlier results on RD^{Rio} TB.^{6,7,10} However, the previously described association of lung cavitory disease,^{7,10} hemoptysis and higher bacillary load with RD^{Rio} TB were not observed in the present study. Possible explanations include sample size, differences between this study and prior studies in the ratio of non-RD^{Rio} LAM and other genotypes in relations to RD^{Rio} strains, nutrition, and social changes occurring in Rio de Janeiro and Brazil in general.

This study was designed with the following power (80–90%, $\alpha = 0.5\%$) and sample size considerations, but was terminated because the anticipated grant did not materialize. The sample size estimated for hemoptysis (RD^{Rio} 30% vs. non-RD^{Rio} 10%) was 250–313, for cavitory disease (RD^{Rio} 40% vs. non-RD^{Rio} 25%) it was 627–783, and for sputum bacillary load 3+ (RD^{Rio} 20% vs. non-RD^{Rio} 10%) it was 627–783. Of note, in the current study, sputum bacillary load 3+ ORs for RD^{Rio} was 1.6 ($P = 0.09$), despite the fact that this study did not attain the anticipated sample size. In addition, in our prior studies in Brazil, the proportion of RD^{Rio} genotype strains was 39% and 37%,^{6,16} while in this study, the proportion of RD^{Rio} genotype strains was 31%. Importantly, the total cohort for our first study in Rio de Janeiro had 310 unique patient isolates, while this study had 272. Lower sample size and/or the lower proportion of isolates with the RD^{Rio} genotype could therefore have affected our ability to demonstrate differences in this study. The present study finding that anorexia was associated with harboring non-RD^{Rio} genotype *M. tuberculosis* may indicate that the current study also had the above limitations, or it may be due to the better nutritional status of the Brazilian population owing to the improved socio-economic conditions in Brazil in the last 10 years.

CONCLUSIONS

Pulmonary TB caused by *M. tuberculosis* RD^{Rio} sublineage in Rio de Janeiro was not clinically distinctive or more severe than TB caused by non-RD^{Rio} *M. tuberculosis* strains in this series of patients. However, this study had several potential limitations that may have contributed to the lack of differences being observed. Larger prospective studies specifically designed to disclose differential clinical characteristics of TB caused by specific *M. tuberculosis* lineages are thus needed. Such studies would allow conclusive or at least stronger causal inferences regarding the clinical specificity of *M. tuberculosis* lineages/sublineages.

Acknowledgments

The authors thank the personnel of the Federal University of Rio de Janeiro University Hospital/Institute of Thoracic Diseases Mycobacteriology Laboratory. This study was supported by National Institutes for Health, USA, grant NIH R 21 (JLH) and by Innovative Approaches for TB Control in Brazil (International Clinical, Operational and Health Services Research U2R TW006885 [JRLES] and R Chaisson [JLH]). The study was also funded by Projetos de Ensino e Extensão/Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior/Conselho Nacional de Desenvolvimento Científico e Tecnológico, Ministry of Science and Technology, Brazil.

References

1. Comas I, Gagneux S. The past and future of tuberculosis research. PLoS Pathog. 2009; 5:e1000600. [PubMed: 19855821]

2. Tsolaki AG, Hirsh AE, DeRiemer K, et al. Functional and evolutionary genomics of *Mycobacterium tuberculosis*: insights from genomic deletions in 100 strains. *Proc Natl Acad Sci USA*. 2004; 101:4865–4870. [PubMed: 15024109]
3. Rocha A, Elias AR, Sobral LF, et al. Genotyping did not evidence any contribution of *Mycobacterium bovis* to human tuberculosis in Brazil. *Tuberculosis*. 2011; 91:14–21. [PubMed: 21106441]
4. Cardoso Oelemann M, Gomes HM, Willery E, et al. The forest behind the tree: phylogenetic exploration of a dominant *Mycobacterium tuberculosis* strain lineage from a high tuberculosis burden country. *PLoS ONE*. 2011; 6:e18256. [PubMed: 21464915]
5. Gomes HM, Elias AR, Oelemann MA, et al. Spoligotypes of *Mycobacterium tuberculosis* complex isolates from patients residents of 11 states of Brazil. *Infect Genet Evol*. 2012; 12:649–656. [PubMed: 21907830]
6. Weisenberg SA, Gibson AL, Huard RC, et al. Distinct clinical and epidemiological features of tuberculosis in New York City caused by the RD^{Rio} *Mycobacterium tuberculosis* sublineage. *Infect Genet Evol*. 2012; 12:664–670. [PubMed: 21835266]
7. Lazzarini LC, Huard RC, Boechat N, et al. Discovery of a novel *Mycobacterium tuberculosis* lineage that is a major cause of tuberculosis in Rio de Janeiro, Brazil. *J Clin Microbiol*. 2007; 45:3891–3902. [PubMed: 17898156]
8. Gutacker M, Smoot J, Migliaccio CAL, et al. Genome-wide analysis of synonymous single nucleotide polymorphisms in *Mycobacterium tuberculosis* complex organisms: resolution of genetic relationships among closely related microbial strains. *Genetics*. 2002; 162:1533–1543. [PubMed: 12524330]
9. Gibson AL, Huard RC, Gey van Pittius N, et al. Application of sensitive and specific molecular methods to uncover global dissemination of the major RD^{Rio} sublineage of the Latin American-Mediterranean *Mycobacterium tuberculosis* spoligotype family. *J Clin Microbiol*. 2008; 46:1259–1267. [PubMed: 18234868]
10. Lazzarini LC, Spindola SM, Bang H, et al. RD^{Rio} *Mycobacterium tuberculosis* infection is associated with a higher frequency of cavitary pulmonary disease. *J Clin Microbiol*. 2008; 46:2175–2183. [PubMed: 18463217]
11. Caws M, Thwaites G, Dunstan S, et al. The influence of host and bacterial genotype on the development of disseminated disease with *Mycobacterium tuberculosis*. *PLoS Pathog*. 2008; 4:e1000034. [PubMed: 18369480]
12. Kent, PT.; Kubika, GP. Public health mycobacteriology. A guide for the level III laboratory. Atlanta, GA, USA: Department of Health and Human Services, Centers for Disease Control and Prevention; 1985.
13. Canetti G, Rist N, Grosset J. Measurement of sensitivity of the tuberculous bacillus to antibacillary drugs by the method of proportions: methodology, resistance criteria, results and interpretation. *Rev Tuberc Pneumol*. 1963; 27:217–272.
14. Kamerbeek J, Schouls L, Kolk A, et al. Detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. *J Clin Microbiol*. 1997; 35:907–914. [PubMed: 9157152]
15. Brudey K, Driscoll JR, Rigouts L, et al. *Mycobacterium tuberculosis* complex genetic diversity: mining the fourth international spoligotyping database (SpolDB4) for classification, population genetics and epidemiology. *BMC Microbiol*. 2006; 6:23. [PubMed: 16519816]
16. de Jong BC, Hill PC, Aiken A, et al. Progression to active tuberculosis, but not transmission, varies by *Mycobacterium tuberculosis* lineage in The Gambia. *J Infect Dis*. 2008; 198:1037–1043. [PubMed: 18702608]
17. Borgdorff MW, Van Deutekom H, De Haas PE, Kremer K, Van Soolingen D. *Mycobacterium tuberculosis* Beijing genotype strains not associated with radiological presentation of pulmonary tuberculosis. *Tuberculosis*. 2004; 84:337. [PubMed: 15207809]
18. van Crevel R, Nelwan RH, de Lenne W, et al. *Mycobacterium tuberculosis* Beijing genotype strains associated with febrile response to treatment. *Emerg Infect Dis*. 2001; 7:880–888. [PubMed: 11747703]

19. Feng JY, Su WJ, Tsai CC, Chang SC. Clinical impact of *Mycobacterium tuberculosis* W-Beijing genotype strain infection on aged patients in Taiwan. *J Clin Microbiol.* 2008; 46:3127–3129. [PubMed: 18596137]
20. Kong Y, Cave MD, Zhang L, et al. Association between *Mycobacterium tuberculosis* Beijing/W lineage strain infection and extrathoracic tuberculosis: insights from epidemiologic and clinical characterization of the three principal genetic groups of *M. tuberculosis* clinical isolates. *J Clin Microbiol.* 2007; 45:409–414. [PubMed: 17166963]
21. Kubica T, Agzamova R, Wright A, et al. The Beijing genotype is a major cause of drug-resistant tuberculosis in Kazakhstan. *Int J Tuberc Lung Dis.* 2005; 9:646–653. [PubMed: 15971392]
22. Thwaites G, Caws M, Chau TT, et al. Relationship between *Mycobacterium tuberculosis* genotype and the clinical phenotype of pulmonary and meningeal tuberculosis. *J Clin Microbiol.* 2008; 46:1363–1368. [PubMed: 18287322]
23. Drobniewski F, Balabanova Y, Nikolayevsky V, et al. Drug-resistant tuberculosis, clinical virulence, and the dominance of the Beijing strain family in Russia. *JAMA.* 2005; 293:2726–2731. [PubMed: 15941801]
24. Agerton TB, Valway SE, Blinkhorn RJ, et al. Spread of strain W, a highly drug-resistant strain of *Mycobacterium tuberculosis*, across the United States. *Clin Infect Dis.* 1999; 29:85–92. [PubMed: 10433569]
25. Iorger TR, Feng Y, Chen X, et al. The non-clonality of drug resistance in Beijing-genotype isolates of *Mycobacterium tuberculosis* from the Western Cape of South Africa. *BMC Genomics.* 2010; 11:670. [PubMed: 21110864]
26. Maree F, Hesselting AC, Schaaf HS, et al. Absence of an association between *Mycobacterium tuberculosis* genotype and clinical features in children with tuberculous meningitis. *Pediatr Infect Dis J.* 2007; 26:13–18. [PubMed: 17195699]
27. Nicol MP, Sola C, February B, et al. Distribution of strain families of *Mycobacterium tuberculosis* causing pulmonary and extra-pulmonary disease in hospitalized children in Cape Town, South Africa. *J Clin Microbiol.* 2005; 43:5779–5781. [PubMed: 16272518]
28. Von Groll A, Martin A, Felix C, et al. Fitness study of the RD^{Rio} lineage and Latin American-Mediterranean family of *Mycobacterium tuberculosis* in the city of Rio Grande, Brazil. *FEMS Immunol Med Microbiol.* 2010; 58:119–127. [PubMed: 19889037]
29. Homolka S, Niemann S, Russell DG, Rohde KH. Functional genetic diversity among *Mycobacterium tuberculosis* complex clinical isolates: delineation of conserved core and lineage-specific transcriptomes during intracellular survival. *PLoS Pathog.* 2010; 6:e1000988. [PubMed: 20628579]
30. Mitchison DA, Wallace JG, Bhatia AL, Selkon JB, Subbaiah TV, Lancaster MC. A comparison of the virulence in guinea-pigs of South Indian and British tubercle bacilli. *Tubercle.* 1960; 41:1–22. [PubMed: 14423002]
31. Palanisamy GS, DuTeau N, Eisenach KD, et al. Clinical strains of *Mycobacterium tuberculosis* display a wide range of virulence in guinea pigs. *Tuberculosis.* 2009; 89:203–209. [PubMed: 19251482]
32. Lopez B, Aguilar D, Orozco H, et al. A marked difference in pathogenesis and immune response induced by different *Mycobacterium tuberculosis* genotypes. *Clin Exp Immunol.* 2003; 133:30–37. [PubMed: 12823275]
33. Horne DJ, Royce SE, Gooze L, et al. Sputum monitoring during tuberculosis treatment for predicting outcome: a systematic review and meta-analysis. *Lancet Infect Dis.* 2010; 10:387–394. [PubMed: 20510279]

Table 1

M. tuberculosis spoligotype families and their frequencies among non-RD^{Rio} and RD^{Rio} TB pulmonary TB patients

Spoligotype	Non-RD ^{Rio} -positive (n = 139) n (%)	RD ^{Rio} -positive (n = 50) n (%)
LAM (Type 1321)	0	1 (2.0)
LAM 1	0	7 (14.0)
LAM 2	0	5 (10.0)
LAM 3	12 (8.6)	0
LAM 3 S/convergent	2 (1.4)	0
LAM 4	5 (3.6)	5 (10.0)
LAM 6	13 (9.4)	0
LAM 9	5 (3.6)	32 (64.0)
Haarlem 1	11 (8.0)	0
Haarlem 3	34 (24.6)	0
Haarlem 4	2 (1.4)	0
U	6 (4.3)	0
U (likely H)	1 (0.7)	0
X1	2 (1.4)	0
X2	2 (1.4)	0
X3	2 (1.4)	0
S	2 (1.4)	0
Beijing	1 (0.7)	0
Beijing Like	1 (0.7)	0
T4-CEU1	3 (2.2)	0
T5_MAD2	1 (0.7)	0
T3	3 (2.2)	0
T2-T3	2 (1.4)	0
T1	29 (20.9)	0

TB = tuberculosis; LAM = Latin American–Mediterranean.

Table 2

Socio-demographic and clinico-radiological features of non-RD^{Rio} and RD^{Rio} pulmonary tuberculosis patients at the time of diagnosis

	Non-RD ^{Rio}		RD ^{Rio}		P value	OR (95%CI)
	Total n	Positive n (%)	Total n	Positive n (%)		
Cases, n	200	200 (100)	72	72 (100)		
Demographic features						
Female sex	200	70 (35)	72	17 (23.6)	0.08	0.57 (0.31–1.1)
Age						
Median [range]	200	41 [18–86]	72	39.0 [19–72]	0.46	—
Median, males	130	44.0	55	39.0	0.01	—
Median, females	70	34.0	17	42.0	0.08	—
Clinical features						
Signs and symptoms at the time of diagnosis						
Cough	195	164 (84.1)	72	58 (80.5)	0.49	0.78 (0.39–1.6)
Sputum	188	129 (68.6)	64	43 (67.2)	0.83	0.94 (0.51–1.7)
Hemoptysis	186	31 (16.7)	65	13 (20.0)	0.54	1.30 (0.61–2.6)
Chest pain	162	80 (49.4)	56	23 (41.1)	0.28	0.71 (0.39–1.3)
Fever	191	112 (58.6)	67	41 (61.2)	0.71	1.1 (0.63–2.0)
Sweats	175	91 (52.0)	57	27 (47.4)	0.54	0.83 (0.46–1.5)
Anorexia	149	76 (51.0)	49	15 (30.6)	0.01	0.42 (0.21–0.84)
Weight loss	184	145 (78.8)	67	55 (82.0)	0.57	1.2 (0.60–2.5)
CXR features at the time of diagnosis*	157		49			
Extension of pulmonary involvement						
Normal CXR		2 (1.3)		0	1.0	0.63 (0.03–13.3)
1 pulmonary lobe		46 (29.3)		21 (42.8)	0.08	1.8 (0.93–3.5)
>1 pulmonary lobe		109 (69.4)		28 (57.1)	0.11	0.59 (0.30–1.1)
Cavitary disease	157	85 (54.1)	49	26 (53.0)	0.89	0.96 (0.50–1.8)
Previous anti-tuberculosis treatment status						
Never treated	194	156 (80.4)	69	59 (85.5)	0.35	1.4 (0.67–3.1)
Relapse after previous treatment (<5 years)	194	24 (12.4)	69	8 (11.6)	0.86	0.93 (0.40–2.2)

	Non-RD ^{Rto}		RD ^{Rto}		P value	OR (95%CI)
	Total n	Positive n (%)	Total n	Positive n (%)		
Body mass index on admission, median	148	19.9	57	20.3	0.50	—
BCG scar	175	115 (65.7)	64	42 (65.6)	0.98	1.0 (0.54–1.80)
TST (>5 mm)	144	108 (75.0)	50	39 (78.0)	0.67	1.2 (0.55–2.5)
Current smoker	195	46 (23.6)	71	23 (32.4)	0.15	1.6 (0.85–2.8)
HIV co-infection	153	14 (9.1)	45	3 (6.7)	0.77	0.71 (0.19–2.6)
Diabetes mellitus	190	26 (13.7)	67	9 (13.4)	0.96	0.98 (0.43–2.2)

* Of 217 films, 11 were excluded due to poor quality reasons and CXR results were not available for analysis in 57.

OR = odds ratio; CI = confidence interval; CXR = chest X-ray; BCG = bacille Calmette-Guérin; TST = tuberculin skin test; HIV = human immunodeficiency virus.

Table 3

Microbiologic features of non-RD^{Rio} and RD^{Rio} pulmonary tuberculosis patients

	Non-RD ^{Rio}		RD ^{Rio}		P value	OR (95%CI)
	Total n	Positive n (%)	Total n	Positive n (%)		
AFB smear on admission						
Spontaneous sputum	140	127 (90.7)	56	50 (89.3)	0.76	0.85 (0.31–2.37)
Induced sputum or BAL	60	34 (57.6)	16	5 (31.25)	0.09	0.33 (0.10–1.08)
Bacillary load on admission *	196 [†]		72			
1–19 colonies		19 (9.7)		10 (13.9)	0.33	1.5 (0.66–3.4)
1+		77 (39.3)		23 (31.9)	0.27	0.73 (0.41–1.3)
2+		52 (26.5)		14 (19.4)	0.23	0.67 (0.34–1.3)
3+		48 (24.5)		25 (34.7)	0.09	1.6 (0.91–2.9)
Median time to growth detection, days	200	(21.0)	72	(21.5)	0.58	—
Culture-positive at 2 months of treatment	57	14 (24.6)	20	5 (25.0)	1.0	1.02 (0.32–3.33)
Drug resistance [‡]						
RMP	193	7 (3.6)	70	1 (1.4)	0.69	0.39 (0.05–3.2)
INH	191	20 (10.5)	70	3 (4.3)	0.14	0.38 (0.11–1.3)
Ethambutol	188	5 (2.7)	69	1 (1.4)	1.0	0.54 (0.06–4.70)
Streptomycin	193	17 (8.8)	70	6 (8.6)	0.95	0.97 (0.37–2.6)
Ethionamide	176	3 (1.7)	61	0	0.57	0.40 (0.02–7.9)
MDR-TB [§]	170	6 (0.3)	61	1 (0.04)	0.46	0.46 (0.05–3.86)

* 1+ = 20–100 colonies; 2+ = 100–200 colonies; 3+ = 201 colonies.

[†] 4 cultures performed using Mycobacteria Growth Indicator Tube 960 were excluded.

[‡] Pyrazinamide susceptibility testing was not performed.

[§] Resistance to both INH and RMP.

OR = odds ratio; CI = confidence interval. AFB = acid-fast bacilli; BAL = bronchoalveolar lavage; RMP = rifampin; INH = isoniazid; MDR-TB = multidrug-resistant tuberculosis.