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Insights into the Origin, Emergence, and Current Spread of a Successful Russian Clone of *Mycobacterium tuberculosis*

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SUMMARY

Mycobacterium tuberculosis variant Beijing B0/W148 is regarded as a successful clone of *M. tuberculosis* that is widespread in the former Soviet Union and respective immigrant communities. Understanding the pathobiology and phylogeography of this notorious strain may help to clarify its origin and evolutionary history and the driving forces behind its emergence and current dissemination. I present the first review and analysis of all available data on the subject. In spite of the common perception of the omnipresence of B0/W148 across post-Soviet countries, its geographic distribution shows a peculiar clinal gradient. Its frequency peaks in Siberian Russia and, to a lesser extent, in the European part of the former Soviet Union. In contrast, the frequency of B0/W148 is sharply decreased in the Asian part of the former Soviet Union, and it is absent in autochthonous populations elsewhere in the world. Placing the molecular, clinical, and epidemiological features in a broad historical, demographic, and ecological context, I put forward two interdependent hypotheses. First, B0/W148 likely originated in Siberia, and its primary dispersal was driven by a massive population outflow from Siberia to European Russia in the 1960s to 1980s. Second, a historically recent, phylogenetically demonstrated successful dissemination of the Beijing B0/W148 strain was triggered by the advent and wide use of modern antituberculosis (anti-TB) drugs and was due to the remarkable capacity of this strain to acquire drug resistance. In contrast, there is some indication, but not yet systematic proof, of an enhanced virulence of this strain.

I am I and my circumstance.
J. Ortega y Gasset, *Meditations on Quixote*

INTRODUCTION

Paradoxically, and contrary to its etymology, a "circumstance" lies within no less than beyond, and the last word in the above famous maxim may well be replaced with a much more earthly one: microbes. During the long course of its evolution, the species *Homo sapiens* has acquired a "second genome," i.e., the human microbiome, defined as the sum of all our resident microbes, which coevolved with their hosts. From the phylogeographic

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point of view, the human microbiome may be regarded as an alternative source of data for deciphering human migrations and origins. Reciprocally, knowledge about human migration and demographics may explain, under certain limitations, the origin and dispersal of human pathogens.

Evolutionary histories of *H. sapiens* and human pathogens are at least partly comirrored and coshaped. However, horizontally/epidemically transmitted pathogens (e.g., influenza virus and HIV) are less likely to mirror the population structure of the human host; instead, their population structures reflect a rapid outburst spreading of particular genotypes. Accordingly, microbe-aided tracing of the events in human history should rely on nonepidemically or mainly latently manifested microorganisms with a family/household mode of transmission and sufficient prevalence in a population. Regarding human tuberculosis (TB), historically short, devastating periods of horizontal epidemic spread have been followed by longer time spans of relatively peaceful coexistence of the surviving human populations and the family/household-transmitted pathogen. Therefore, *Mycobacterium tuberculosis* is a suitable candidate for tracing human migrations, since remarkably useful features of *M. tuberculosis* include (i) a high prevalence (~30%) of latent tuberculosis in humans, and hence a mainly family/household mode of transmission during most of human history; and (ii) an extreme rarity of lateral gene transfer, and hence less risk of diffusing the phylogeographic signal.

Genome Markers of *M. tuberculosis*

The *M. tuberculosis* genome contains various insertion sequences (IS*6110* is the most widely used) and repeat loci that have been used widely to study the genetic diversity of the species. The most frequently used of the latter include one CRISPR (historically named a direct repeat [DR] [1]) locus and multiple variable-number tandem-repeat (VNTR) loci (Fig. 1).

The CRISPR locus in *M. tuberculosis*, as in other bacteria, consists of minisatellite alternating exact direct repeats and variable spacers (Fig. 1A). Its evolution in *M. tuberculosis* currently occurs via neutral consecutive deletions of either single units or contiguous blocks (2). A method to study variation in the CRISPR locus in *M. tuberculosis* is named spoligotyping, which in its classical and usually macroarray-based format detects the presence or absence of 43 particular spacers (Fig. 1A). A more comprehensive analysis of the CRISPR locus relies on 68 different spacers, which gives some added discriminatory value (2). As a whole, the method is simple and portable but lacks sufficient discriminatory power. For this concern, more discriminatory methods of *M. tuberculosis* strain typing deal with more polymorphic IS*6110* and VNTR loci.

IS*6110* restriction fragment length polymorphism (IS*6110*-RFLP) typing is based on analysis of the insertion sequence IS*6110*, which is found in most isolates of the *M. tuberculosis* complex (Fig. 1D). Until relatively recently, this was the most widely applied and standardized molecular typing method for *M. tuberculosis* complex isolates. The method was considered to be the most discriminatory and was thus the most widely used method in epidemiological studies (3). Unfortunately, it is cumbersome and time-consuming and requires large quantities of DNA, and interlaboratory comparison of results is difficult.

Mycobacterial interspersed repetitive unit-VNTR (MIRU-VNTR) loci are scattered throughout the genome, and isolates can be typed based on the number of copies of repeated units (4) (Fig. 1B). Implementation of the large number of carefully evaluated loci has been expected to achieve a high level of discrimination. Since 1998, the VNTR typing of *M. tuberculosis* has undergone a remarkable development. Whereas the first scheme comprised only 6 exact tandem-repeat loci (5), a more recently implemented but already classical MIRU set involved 12 loci (4), and finally, the new format for MIRU typing includes 24 loci (6). The necessity of further adjusting this scheme to particular settings by adding so-called hypervariable loci was recognized most recently (7–9). This method was shown to possess a higher discriminatory power than spoligotyping and, depending on the number of VNTR loci tested, a power roughly similar to that of IS*6110*-RFLP typing (6, 7). The other apparent advantage of the VNTR approach (compared to IS*6110* typing) is its portable format, and hence its easy international exchange and use in database development.

A number of other molecular markers/methods, such as single-nucleotide polymorphisms (SNPs), large deletions (regions of difference [RD]), and whole-genome, new-generation sequencing, should be mentioned. They vary in discriminatory power, although they demonstrate a clear potential to reconstruct reliable phylogenies and may aid in high-resolution epidemiological investigations (10). However, they are not relevant to the topic of this review and thus are not discussed further here.

Population Structure and Clinical Relevance

It is very common yet true saying that *M. tuberculosis* is a major human killer. However, a closer look at the population structure of the pathogen reveals that some of its lineages show a greater capacity to spread and cause active disease. Consequently, efficient application of modern antituberculosis treatment regimens is hampered by dissemination of such *M. tuberculosis* strains. *M. tuberculosis* has a clonal population structure, and several genetic families have been identified within this species, e.g., East-African-Indian, CAS/Delhi, Beijing, Haarlem, Manu, and LAM. Initially endemic within specific geographical areas, some of these families have become ubiquitous. An example of such an omnipresent lineage is the Beijing family/genotype. It was identified for the first time in the Beijing area of China (11), but strains of this lineage are now found on all continents, although at different rates, and thus may be defined as endemic, epidemic, or sporadic (12, 13).

In addition to the lineage/family-associated strain properties, it has been recognized that some of the clonal clusters (sublineages and subgenotypes) within the same family may be more transmissible than others. In particular, while the Beijing genotype as a whole is frequently (but not always) marked by drug resistance and hypervirulence (reviewed in reference 14), some of its variants demonstrate even more remarkable pathogenic properties, especially in areas with a high burden of drug-resistant TB (15). There are several classifications of Beijing genotype strains, in particular by subdividing them into ancient or ancestral and modern groups (16–18), which are suggested to differ in mechanisms of adaptation to drug-induced selective pressure (19). In China, the modern Beijing strains are more likely than the ancient sublineages to be clustered (20), and an increasing circulation of the modern Beijing sublineage was recently demonstrated in Japan (21).

While discrimination into modern and ancient Beijing strains provided a rough, phylogenetically meaningful subdivision, high-resolution typing permitted the definition of more homogeneous

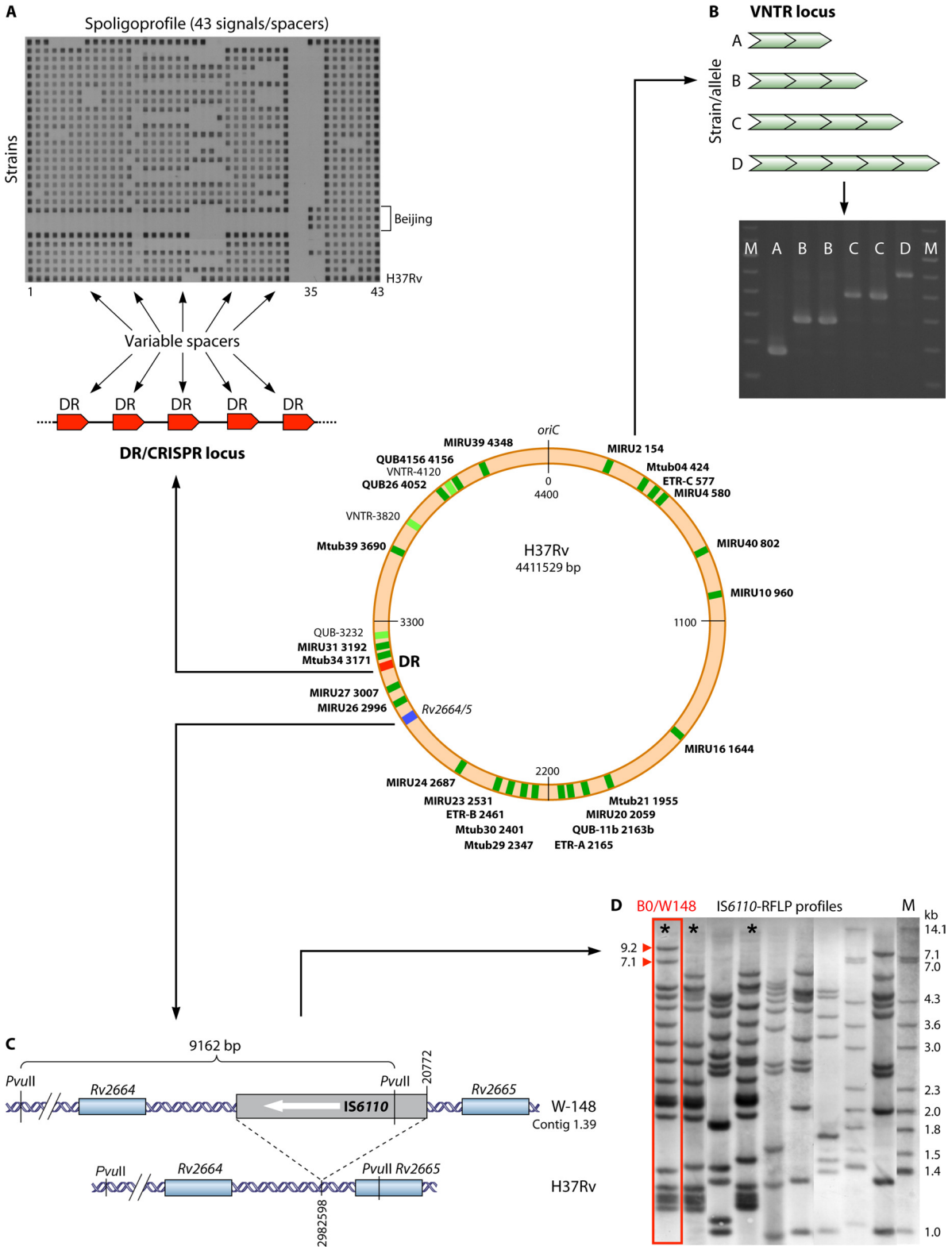

FIG 1 Positions of particular genomic loci on a circular map of *M. tuberculosis* H37Rv, along with their structure and analysis. (A) DR/CRISPR locus and 43-spacer-based spoligotyping. (B) Multiple VNTR loci, including 24 MIRU-VNTR (in bold) and 3 hypervariable loci, and their analysis using agarose gel electrophoresis. M, 100-bp ladder. (C) *Rv2664-Rv2665* region in H37Rv and W-148 strains. (D) IS*6110*-RFLP typing, with hybridization of PvuII-digested DNAs of *M. tuberculosis* strains with an IS*6110*-derived probe. Beijing genotype strains are shown by asterisks. The characteristic double band in the profile of strain B0/W148 is shown by short arrows. M, strain Mt14323, used as a molecular size marker.

clonal clusters or strains of clinical and epidemiological interest. Notorious examples include strain W, which caused the multidrug-resistant TB (MDR-TB) epidemic in New York City in the early 1990s (22); the highly transmissible Beijing strain in Gran Canaria Island, whose circulation is still ongoing (23, 24); and the Russian strain Beijing B0/W148 (25–28), the subject of this review.

Hypothesis and Questions

A clonal group named B0 or W148 (27, 28) has been reported for about one-fourth of the Beijing genotype isolates in different parts of Russia and the former Soviet Union and in Russian immigrants in the United States and Europe (25–31). It has been hypothesized that the Beijing B0/W148 clone spread historically recently throughout the former Soviet Union due to its special pathogenic properties and thus may represent a successful clone of *M. tuberculosis* (8). However, an integral picture of the time and location of origin, evolutionary history, and biological properties of the Beijing B0/W148 strain remains obscure. Did it originate within the borders of Russia or the former Soviet Union, or was it imported from abroad, for example, from East Asia, where the Beijing genotype is a prevalent lineage, or from other parts of Asia? What was the driving force of its dissemination; more specifically, what human movement served, unwittingly, as a vehicle? What pathobiological properties underlie its spread? I tried to answer these questions by searching the published literature for the presence and properties of Beijing B0/W148 strains in local populations of *M. tuberculosis*. A systematic and critical review, reanalysis, and new analyses of these data are presented below.

SAMPLING AND METHODS

Selection of Studies

The *M. tuberculosis* Beijing family isolates were defined based on spoligotyping: isolates showing hybridization to at least three of spacers 35 to 43 and an absence of hybridization to spacers 1 to 34 were defined as having the Beijing genotype (32) (Fig. 1A).

M. tuberculosis Beijing B0/W148 isolates were defined based on visual similarity of their IS*6110*-RFLP profiles to the prototype profile (8, 27, 28) (Fig. 1D). One should note that no freely accessible database of IS*6110*-RFLP profiles exists and that the search for profiles was done directly in the original articles. More details on genetic features of B0/W148 are provided below.

For literature search, the Google, Google Scholar, Scopus, and Medline search engines and databases were used. The primary keywords used were "tuberculosis," "Beijing genotype," "B0," "W148," "IS6110," "fingerprinting," "spoligotyping," "typing," and country names, in English and Russian.

The primary definition of the W148 clone was based on the IS*6110*-RFLP profile; in this sense, the inclusion of data on IS*6110*-RFLP profiles was the key feature of the article to be considered for further analysis. In fact, very few studies mention this clone in the abstract, and searches of raw data and other analyses were performed directly on the information found in or extracted from the full-text articles. A per-country search was based on the general perception of the geographic specificity of B0/W148 to the former Soviet Union, i.e., the search of IS*6110*-RFLP profiles in areas beyond Eurasia was representative but not exhaustive. The B0/W148 isolates recovered from immigrants from the former Soviet Union in Europe and the Americas make a special case and were studied separately: these studies were not included in geographical mapping (Fig. 2), but information on patients' country of origin was used in some kinds of analysis (see Table S1 in the supplemental material). Data on the Beijing genotype as a whole are shown only for Eurasia, where B0/W148 is circulating, and only for locations with available data on IS*6110*-RFLP profiles.

Thus, in total, 63 studies were retained based on visual inspection of the content and were used for the different aspects of analysis presented in this review (15, 25–27, 29–31, 33–88) (see Table S1 in the supplemental material).

The limitations of this review should be acknowledged: as it was based on published studies, it depended on their own limitations and potential biases. First, not all studies used all molecular methods/markers necessary for identification of the B0/W148 strain and its further subtyping. Second, only a few studies made a specific focus of the phenotypic features of the B0/W148 strain, and most studies did not show data on B0/W148 alone but as part of the Beijing family. Third, not all studies provided data on individual isolates/patients, so it was not possible to extract the precise strain/patient-specific details.

All available publications dealing with genotyping data and published since the early 1990s were (re)analyzed in order to establish the rate of Beijing B0/W148 in local populations of *M. tuberculosis* (see Table S1 in the supplemental material). The obtained information was further interpreted in light of the meaningful clinical, epidemiological, and phenotypic strain/patient properties. The summarized data are shown in Table S1 and in Fig. 2. In spite of a bias of some studies, certain special settings, such as prisons, offered additional interesting clues to a better understanding of the epidemiology of the Beijing B0/W148 variant.

Methods of Analysis

Genotyping. Typing data obtained by classical and more recent methods, i.e., IS*6110*-RFLP typing, spoligotyping, and MIRU-VNTR typing, performed according to international protocols (6, 89, 90), were considered in the analysis.

The international databases MIRU-VNTRplus (www.miru-vntrplus.org) (91) and SITVIT Web (http://www.pasteur-guadeloupe.fr:8081/SITVIT_ONLINE/) (92) were consulted for attributing international codes to the MIRU-VNTR profiles.

Statistical and phylogenetic analysis. The Hunter-Gaston index (HGI) was used as an estimate of the diversity/discriminatory power of a molecular marker/method. The HGI was calculated using the following formula:

$$\mathrm{HGI} = 1 - \frac{1}{N(N-1)} \sum_{j=1}^{s} n_j(n_j - 1)$$

where N is the total number of strains in the typing scheme, s is the total number of distinct patterns discriminated by the typing method, and n_j is the number of strains belonging to the jth pattern (93).

The minimum spanning tree (MST) and neighbor-joining tree based on VNTR types were built using the PAUP* package (94) and the PARS routine of the PHYLIP 3.6 package (95), respectively. VNTR alleles were treated as categorical variables.

Meta-analysis was carried out using the Review-Manager 4.3 (Oxford, United Kingdom) and STATA, version 10 (Stata Corp.), programs. The inconsistency index (I^2) was calculated, and a χ^2-based Q-statistic test was performed to evaluate between-study heterogeneity (96, 97); I^2 values of >50% and P values of <0.1

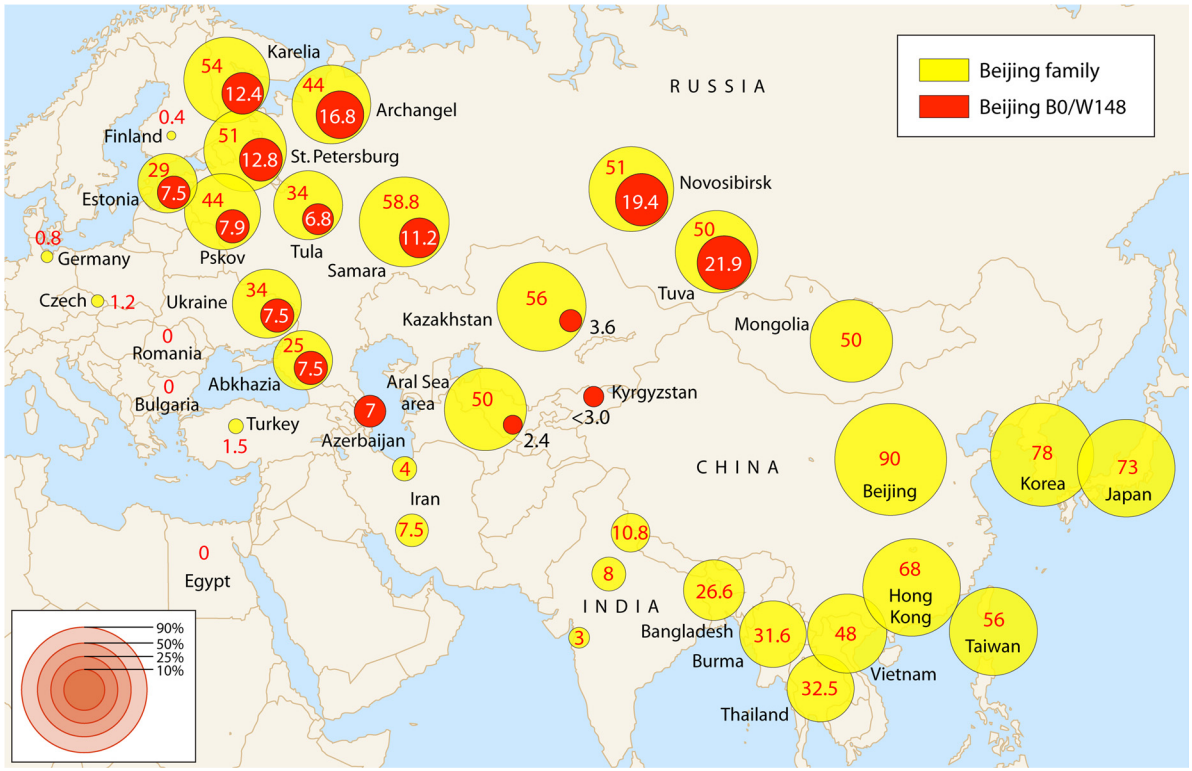

FIG 2 Global distribution of *M. tuberculosis* Beijing genotype and Beijing B0/W148 strains in autochthonous populations in Eurasia. Data are not shown for (i) prison settings and (ii) immigrant communities in Europe. Data on the Beijing family as a whole are shown only if the frequency of the B0/W148 strain could be verified by an IS*6110*-RFLP or multiplex PCR method (33). For Azerbaijan and Kyrgyzstan, no information on the Beijing family rate is shown, and estimated rates of B0/W148 prevalence are shown (see Table S1 in the supplemental material for details). Numbers in/at circles show percentages of the Beijing family and B0/W148 strain in the *M. tuberculosis* population in a given area; circle sizes are proportional to these percentages.

indicated significant heterogeneity. A Z test was used to test the significance of the odds ratio (OR) (P values of <0.05 were considered significant). Begg's rank correlation method was used to statistically assess publication bias (no bias was present if the Z value was <1.96 and the P value was >0.05) (98).

Comparison with human history. Searches for major flows of human migration and historical links between the areas targeted in this work were done by searching Google, Entrez Pubmed (http://www.ncbi.nlm.nih.gov/sites/entrez), and Russian Demoscope (http://demoscope.ru/weekly) search resources. The primary keywords used were "human migration," "tuberculosis," "history," "phylogeography," "coevolution," and relevant geographic names used alone and in combination, in English and Russian. This procedure was followed by further sorting and mining of the large body of retrieved information and, if necessary, additional searching using more specific keywords covering bilateral relations between particular regions and countries. Although this method is neither exhaustive nor quite systematic, a quantitative approach to comprehensively study large events in human history does not yet exist, to the best of my knowledge.

GENETIC FEATURES OF BEIJING B0/W148

The Beijing B0/W148 variant was initially defined using IS*6110*-RFLP typing (25–28) and is marked by a characteristic double band (7.1 and 9.2 kb) in the upper part of the profile (Fig. 1D). Strains with the Beijing B0/W148 sensu stricto profile and similar profiles sharing the same characteristic double band were more recently defined as the B0/W148 cluster (8). Thus, IS*6110*-RFLP typing is, by definition, the "gold standard" method for defining B0/W148 cluster isolates.

B0/W148 isolates have a nine-signal 43-spacer spoligotyping profile that is characteristic of the whole Beijing family (16, 27, 48, 53) (Fig. 1). More comprehensively, based on 68-spacer spoligotyping, the CRISPR locus in B0/W148 isolates consists of 15 units, although this was shown only for isolates from northwestern Russia (16) and is a feature common to other Beijing genotype variants. A discussion about "pseudo-Beijing" strains (99) is beyond the scope of this article.

The evolution of the VNTR loci of the Beijing B0/W148 isolates in Russia is discussed in more detail below. In St. Petersburg, in northwestern Russia, most of these isolates had the 24-MIRU profile 24423335264442517335372 3 (8); the loci are listed according to their clockwise order in the genome (Fig. 1; see Table S2 in the supplemental material). In the international MIRU-VNTRplus database, this 24-locus profile is designated 100-32. Interestingly, this profile also falls within the 12-MIRU type M11 (digital profile 223325173533 [17, 33]), also named MIT17 (SITVIT Web database), which is the largest type in East Asia and the second (but not first!) largest in Russia (13, 100). Three hypervariable loci, QUB3232, VNTR3820, and VNTR4120, were characterized by 14, 14, and 10 copies, respectively, in most of the B0/W148 cluster isolates in the St. Petersburg area of Russia (see Table S2). The presence of seven copies each of MIRU26 and QUB26 was initially suggested to be specific for B0/W148 in northwestern Russia (8).

However, in Novosibirsk, Siberia, the B0/W148 isolates had mainly 8 as well as 6 and 7 copies of QUB26 (44), and the use of this two-locus signature to identify the B0/W148 cluster isolates is apparently limited to the St. Petersburg area of Russia. In summary, although the aforementioned profile 100-32 was prevalent among these strains in St. Petersburg, no single consensus 24-MIRU profile exists for B0/W148.

A complete genome sequence of the first *M. tuberculosis* strain of the B0/W148 cluster was released in 2011 (strain W-148; GenBank accession no. ACSX00000000.1). This was an extremely helpful achievement toward a more in-depth study of the strains of this cluster and a better understanding of their pathogenomics. In particular, this opened new perspectives for the search of new molecular targets for rapid diagnostics for this variant. IS*6110*-RFLP typing is the gold standard method for detecting B0/W148 cluster isolates; however, it is admittedly cumbersome and time-consuming. *In silico* analysis of the complete genome sequence of strain W-148 helped to identify positions and surrounding sequences of IS*6110* insertions (33). The reversed IS*6110* in contig 1.39 (positions 19418 to 20772) was found located within the 9,162-bp PvuII digestion fragment, which corresponded to the largest band of the B0/W148 IS*6110*-RFLP profile (Fig. 1C versus D). This insertion corresponds to positions 2982598 and -9 in the complete genome of strain H37Rv (GenBank accession no. NC_000962.2), in a short intergenic region between the *Rv2664* and *Rv2665* genes. This intergenic region is also both intact and identical in all other published complete genomes of *M. tuberculosis* complex strains of different genotypes available in GenBank. The only exceptions are (i) *M. bovis* and *M. bovis* BCG strains with an A → G substitution 179 nucleotides upstream of this IS*6110* insertion in strain W-148 (position 2935950 in the complete genome sequence of *M. bovis* BCG strain Pasteur 1173P2 [GenBank accession no. NC_008769.1]) and (ii) *M. canetti*, in whose genome no similarity was found. Both the *Rv2664* and *Rv2665* genes encode hypothetical proteins of unknown function ("hypothetical protein" and "hypothetical arginine-rich protein," respectively) and are located within the phiRv2 prophage. Their functions remain unknown, even after a recent reanalysis and reannotation of functions for the hypothetical open reading frames (ORFeome) of the *M. tuberculosis* genome (101). Therefore, whether this particular IS*6110* insertion has a biological meaning and/or confers some hypothetical advantage is unclear.

Whatever its function, this *Rv2664-Rv2665*::IS*6110* insertion was used as a target for a multiplex PCR assay to detect the B0/W148 strain (33). This method was proven to be sensitive and specific and was recommended for simple and reliable detection and surveillance of B0/W148 isolates in areas of epidemic circulation, such as Russia and other countries of the former Soviet Union, and in areas receiving immigrants from these countries. It will also be interesting to apply it to retrospective strain or DNA collections and archival samples from different locations. To date, only one study has used this novel method to assess the prevalence of B0/W148 strains in retrospective strain collections representing Russia, Belarus, China, Vietnam, and Brazil (33).

PATHOBIOLOGY OF BEIJING B0/W148

The pathobiology of microorganisms encompasses those biological properties that may cause, under certain host/pathogen/environment-related conditions, pathological changes in the host organism and/or host population. An understanding of pathobiology is crucial for elucidating the driving forces underlying the tempo and mode of dissemination of a particular strain or clone.

Virulence and Transmissibility

Pathological features. To date, a sufficient number of studies focusing on the Beijing family have analyzed patient-related information and pathological features, such as the course and particularities of the disease progression (duration and severity of disease, febrile response, and disease site, e.g., pulmonary versus extrapulmonary TB), treatment outcomes (such as failure or relapse), patient age (younger versus older), BCG vaccination status, etc. Unfortunately, this kind of clinically meaningful data is extremely scarce for the Beijing B0/W148 strain.

Contrasting pulmonary versus spinal TB, one very recent study in St. Petersburg analyzed the genetic diversity of *M. tuberculosis* strains from adult TB spondylitis patients coming from different Russian regions (35). In particular, a 74.8% rate of prevalence of the Beijing family in the studied population was found (compared to an average 50% rate among pulmonary TB patients across Russia [16, 27, 36–48]). At the same time, the rate of B0/W148 in the Beijing population was 30% (35). Accordingly, when recalculated, the rate of B0/W148 prevalence was 22.4% of all *M. tuberculosis* isolates from TB spondylitis patients, which is double the 12.8% average rate of B0/W148 prevalence in St. Petersburg (Fig. 2; see also Table S1 in the supplemental material]). BCG vaccination, which is recognized to best protect against disseminated TB (although indeed in children [14, 102, 103]), is obligatory in Russia. In my opinion, this finding suggested an association of B0/W148 and spinal TB and thus can be interpreted as a propensity of this strain to cause extrapulmonary TB disease in the BCG-protected population, i.e., as an indication of increased virulence.

Animal and macrophage-based studies. Understanding the transmissibility of a strain, in particular that of an *M. tuberculosis* strain, may perhaps be achieved through gaining knowledge about its virulence, manifested through host-pathogen interactions at the individual and population levels. In this regard, studies using animal models and macrophage-based models may provide a relatively direct proof of virulence/transmissibility properties.

Use of animal models of TB has previously highlighted interstrain variations, and although these findings cannot be extrapolated directly to human infection, they have aided in the study of the pathogenesis and manifestation of the disease. Animal model studies have shown that highly prevalent and clustered strains are more virulent than unclustered sporadic isolates and that highly virulent strains can induce nonprotective immune responses, with some correlation with clinical and epidemiological characteristics (104). Macrophages are a primary target of *M. tuberculosis*, and the ability of the organism to multiply rapidly within these cells is important for pathogen survival. A number of properties have been linked with highly virulent mycobacteria, such as the capacity to kill infected macrophages by cell necrosis, favoring bacterial survival and liberation (105), and the induction of a low level of proinflammatory tumor necrosis factor alpha (TNF-α) and a high level of inhibitory inerleukin-10 (IL-10) production by infected macrophages (106).

It should be noted that differently designed macrophage-based studies produced concordant results and demonstrated an increased virulence of the B0/W148 variant compared to other Beijing variants (15, 107, 108). A comparison of Russian strains of

different genetic families demonstrated that Beijing genotype isolates were more viable than LAM and Ural strains in a murine macrophage model, irrespective of the infecting dose. Interestingly, B0/W148 isolates showed even higher viability in murine macrophages than that of other Beijing strains (108, 109).

A comparative study of MDR Beijing genotype strains from Russia and Brazil found that Russian B0/W148 isolates demonstrated an increased fitness and growth in THP-1 macrophage-like cells (15). The Beijing strains from Russia (including B0/W148 strains) were significantly more cytotoxic than other Beijing strains, and they induced a distinct pattern of cytokine expression favoring immunosuppression: a reduction over time of TNF-α production and a high level of IL-10 secretion. In contrast, MDR Beijing isolates from Brazil showed reduced fitness and a lack of elevated IL-10 production.

Unlike macrophage-based studies, studies using animal models generated contrasting results by two Russian groups (109–111). Vishnevskiĭ et al. (110) demonstrated an increased virulence of the B0/W148 isolates in noninbred albino mice. Their *M. tuberculosis* isolates did not show a genotype versus virulence/viability correlation, but there was a lower rate of decreased virulence in multidrug-resistant isolates of the Beijing B0/W148 genotype than in the isolates of other genotypes (110). A limitation of the study was a control group that included non-Beijing genotypes but not other Beijing family strains. In contrast, a more recent Russian study revealed a heterogeneity of the Beijing genotype isolates in terms of inbred C57BL/6 mouse survival and a lack of special virulence properties of the B0/W148 isolates compared to other Beijing isolates and isolates of other genotypes (109, 111).

Indirect indications of the increased transmissibility of a strain may include (with certain important limitations) a high level of clustering as proof of ongoing active transmission, increased circulation in prison versus community settings, an increasing prevalence in the total population in the survey area, and involvement in an outbreak. This kind of information may be gained through epidemiological studies. Below I review and discuss the available evidence related to these issues.

Clustering. A clustering rate (when calculated based on high-resolution typing data) may serve to estimate transmissibility, especially in the case of clustered MDR isolates from newly diagnosed patients. However, it is difficult to apply this directly to B0/W148 versus other Beijing variants based on IS*6110*-RFLP typing data because the B0/W148 cluster is, by definition, a cluster of strains with similar profiles dominated by the B0/W148 sensu stricto profile. In the Archangel civilian study in northern Russia, the B0 cluster included two IS*6110*-RFLP profiles that differed in a single band and were shared by 5 and 15 isolates (38). Likewise, the Archangel prison study showed only 3 B0 cluster profiles, shared by 19, 4, and 1 isolate (39). In the Abkhazia study, 23 B0/W148 isolates included 14 B0/W148 sensu stricto isolates, 2 small clusters of 2 isolates each, and 5 singletons, all of which were closely related (51).

Meta-analysis is known to increase the effective sample size by pooling of data from individual studies, thereby enhancing the statistical power to assess an association. A meta-estimation of the above clustering data (as a measure of recent transmission) was performed through comparison of the rates of IS*6110*-RFLP-clustered isolates among all Beijing B0/W148 cluster isolates (cases) versus other Beijing genotype strains (controls) (Fig. 3). A funnel plot was applied for comparison of cases versus controls in an OR analysis; Begg's test provided no evidence of funnel-plot asymmetry ($Z = 0.13$; $P = 0.9$) (Fig. 3B). The studies were not heterogeneous but differed in study design, so both fixed- and random-effect models were tested. As a result, the overall effect was found to be significant for both models (Fig. 3A), which confirms an increased clustering of the B0/W148 cluster isolates, thus suggesting their enhanced capacity to be transmitted. While caution is required in interpretation of this result due to the possibility of false clustering by IS*6110*-RFLP typing, such an error would probably equally influence both groups (B0/W148 and other Beijing strains) and hence would not probably produce a significant effect on the outcome.

VNTR typing data (i.e., VNTR-based discrimination within the B0/W148 cluster) might be helpful to more adequately assess the true level of clustering, although this kind of information is limited with regard to B0/W148 cluster isolates. A study in St. Petersburg demonstrated that the B0/W148 cluster is a true monophyletic cluster by both IS*6110*-RFLP typing and high-resolution 27-locus VNTR typing that included 24 MIRU and 3 hypervariable loci (8). This was not the case for the other major Russian IS*6110*-RFLP variant of the Beijing genotype, named A0, which was distributed in different and distant branches of the same VNTR dendrogram (8). A lower level of VNTR diversity for B0/W148 than for A0 cluster strains should also be noted (HGI = 0.54 versus 0.87). Furthermore, a star-like VNTR-based network (Fig. 4) was suggested to mirror a recent/outburst dissemination of these strains in the survey area, i.e., northwestern Russia (8). Indeed, a large core type is linked to almost all other small types by a single locus variation, remarkably, involving different loci. Consequently, from the phylogenetic point of view, the B0/W148 cluster may indeed represent a successful clone of *M. tuberculosis*.

Bacterial population growth. An increasing frequency of a genetic variant in the total local population over time may mirror its increased transmissibility (if confounding factors such as human migration are ruled out). For example, Wirth et al. (112) applied Bayesian statistics to the 24-locus VNTR diversity data in order to estimate the change of effective population size of the different lineages within *M. tuberculosis*. As a result, they demonstrated that a human population explosion in the last 200 years was the primary reason for growth of all lineages, but in the case of the Beijing genotype, the estimated growth rate was higher than the average for other lineages; this finding was interpreted as proof of the enhanced transmissibility of the Beijing genotype (112).

Unfortunately, this kind of data is relatively abundant for the Beijing genotype as a whole but rather limited for the B0/W148 variant. For example, the proportion of Beijing genotype strains among MDR strains from Germany has changed markedly, from 19.2% in 1995 to 58.3% in 2001; however, a clear role of immigration from the countries of the former Soviet Union has been suggested (30), and thus this population growth of the Beijing genotype in Germany was due to an influx of the human population, not to increased transmission of this strain. In Novosibirsk (Siberia, Russia), the data on samples from 2000 and 2009 did not show a difference in the prevalence rates of the Beijing genotype in the local population, with values of 51.5 and 47%, respectively (45, 47). However, information on the Beijing B0/W148 variant in the above studies was available only for a single time point, and it was not possible to trace its change over time.

The data on St. Petersburg since the early 1990s show no visible increase in the rate of B0/W148 in the Beijing genotype popula-

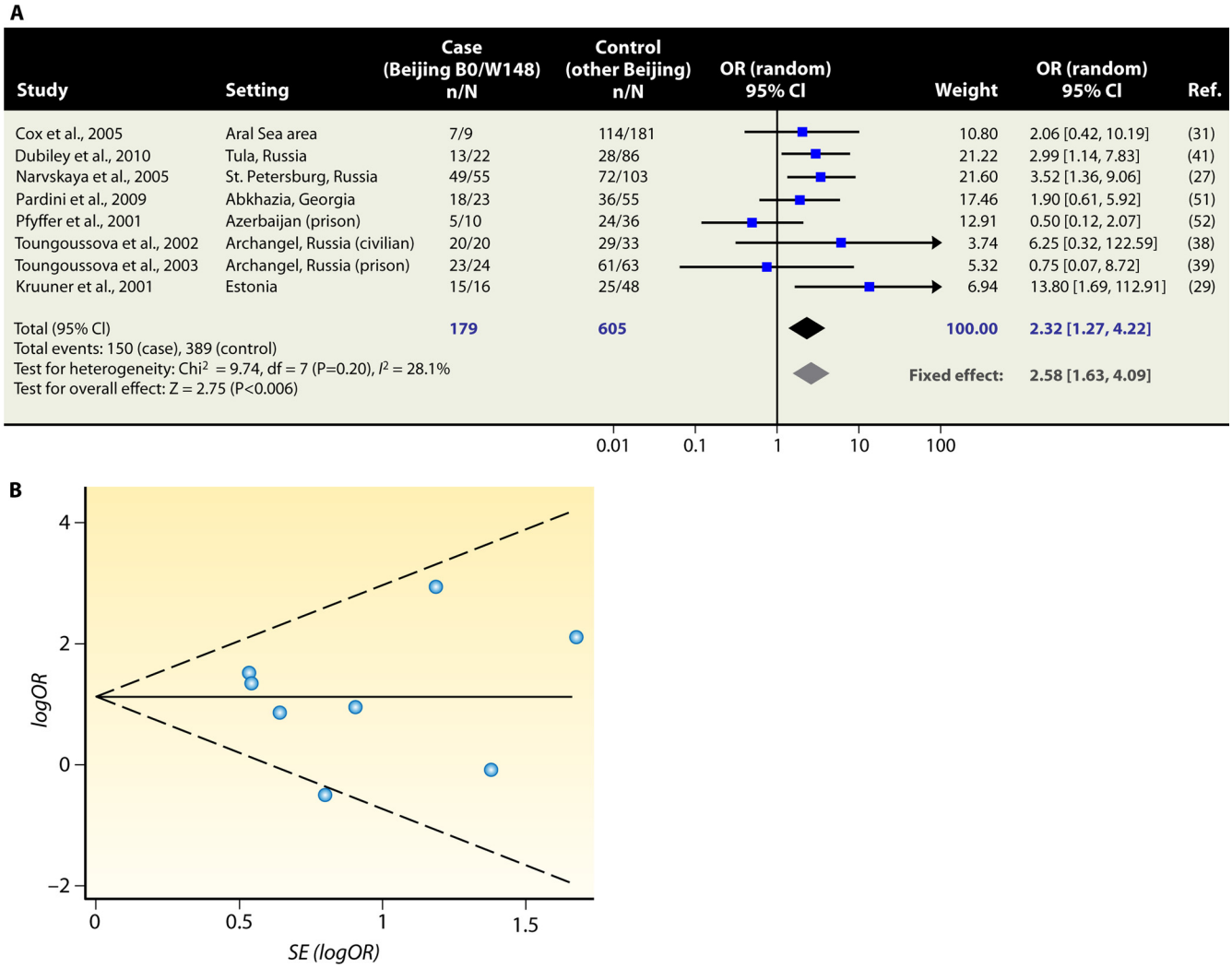

FIG 3 Meta-analysis of the association between *M. tuberculosis* Beijing B0/W148 and clustering, measured as the rate of IS*6110*-RFLP-clustered isolates among all isolates in a given group. Error bars indicate 95% confidence intervals (95% CI). Solid squares represent each study in the meta-analysis. The solid black diamond represents the pooled OR; the gray diamond shows the fixed-effect model pooled OR (in a test for overall effect, $Z = 4.03$ [$P < 0.0001$]). Both civilian and prison samples were considered for the study of Dubiley et al. (41), and drug-resistant strains were considered for the study of Krüüner et al. (29). (B) Begg's funnel plot with pseudo-95% confidence limits. The references in the figure correspond to references 31, 41, 27, 51, 52, 38, 39, and 29.

tion, with rates of 26.7% in 1993 to 1995, 24.3% in 1997 to 2002, and 28.8% in 2008 to 2010 (27, 33, 34), or in the total *M. tuberculosis* population; these studies were carried out in the same setting (Hospital of the Research Institute of Phthisiopulmonology) and thus are comparable. In contrast, the rate of B0/W148 increased over time from 2003 to 2005 in Abkhazia (51). As no significant change in population size and structure was reported for Abkhazia for the years 2003 to 2005 (113, 114), the data of Pardini et al. (51) may be considered proof of increasing circulation of the B0/W148 MDR strain due to its increased transmissibility. On the other hand, demographic data on St. Petersburg (1996 to 2010) show no significant change in population size (115), thus confirming a lack of population growth of the B0/W148 strain over a long survey period, and hence a lack of an enhanced capacity to be transmitted.

Circulation in special settings. Other, indirect evidence of transmissibility may rely on the involvement of a strain in nosocomial or community outbreaks or an enhanced circulation of a strain in prison versus civilian settings. An argument that a strain may happen to be in the right place and right time and thus just coincidentally cause an outbreak is reasonable but not exactly correct under particular conditions of the local population structure of the pathogen. The Beijing genotype comprises up to 90% of isolates in the city of Beijing and surrounding provinces in North China (11, 19), and it would hardly be justified to investigate if a Beijing strain is more responsible than other strains for outbreaks, including nosocomial outbreaks, in this area. In contrast, the Beijing genotype is found at a 40 to 60% frequency across Russian regions, which is far from the overwhelming dominance. Furthermore, other genetic families of *M. tuberculosis* in Russia, such as the LAM family, were suggested to be linked to MDR (41). An increased circulation of Beijing strains (compared to strains of other *M. tuberculosis* genotypes) in prison environments has been demonstrated in most, but not all, settings (see reference 36 and references therein).

Thus, the Russian *M. tuberculosis* population is heterogeneous

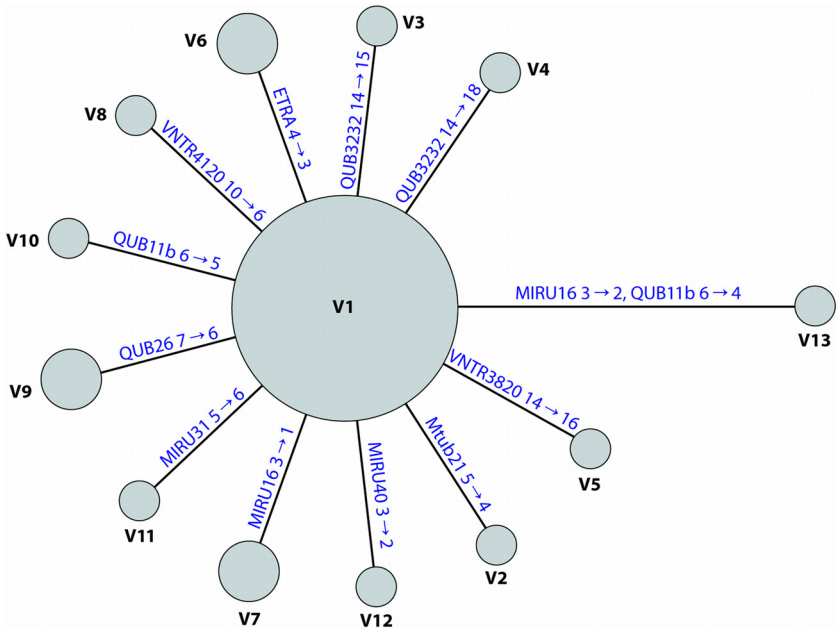

FIG 4 Minimum spanning tree of the VNTR types of B0/W148 cluster isolates from St. Petersburg and other locations in northwestern Russia, based on the combined use of 24 MIRU-VNTR and 3 hypervariable VNTR loci. Circle size is roughly proportional to the number of strains. VNTR digital profiles are shown in Table S2 in the supplemental material. The 24-MIRU component of the central type V1 corresponds to profile 100-32 in the MIRU-VNTRplus database. (Adapted from reference 8.)

without an overwhelming predominance of a single *M. tuberculosis* strain in any setting. Nonetheless, it was the B0/W148 strain that caused a nosocomial outbreak in the above-mentioned TB hospital in St. Petersburg (116); this outbreak concerned one index case (TB patient) and four contacts (three TB patients and a nurse). At the same time, to the best of my knowledge, no other studies supported by classical epidemiological investigations on outbreaks or chains of transmission caused by B0/W148 or other strains in Russian settings have been published. Therefore, it is hardly possible to make a statistically robust conclusion about increased transmissibility of the B0/W148 strain (compared to other Beijing family strains) based on this kind of data only.

On the other hand, transmission under overcrowded and stress-causing prison conditions makes a special case of continuous outbreak. The prisons in the countries of the former Soviet Union in the 1990s were marked by overcrowding, thus facilitating transmission of airborne pathogens. A comparison of prison versus civilian settings may thus help to elucidate the issue of transmissibility of a strain.

It is noteworthy that one of the first papers that drew attention to the *M. tuberculosis* B0/W148 variant, published in 1999, concerned prison settings in Siberia: the B0/W148 strain was found in 5 patients coming from 5 different prisons in the Kemerovo oblast (117). A very high notification rate of TB due to a single clone, namely, B0/W148, was reported for a West Siberian prison, as this strain was recovered from 190 prisoners who were newly diagnosed TB patients in 1998 to 2000 (28). The spread of B0/W148 in the Siberian prison was suggested to be due partly to an ineffective public health infrastructure, the lack of a system for drug susceptibility testing, and inadequate treatment of drug-resistant TB (118). At the same time, nothing was reported about the rate of B0/W148 in the corresponding local civilian population, and it is not possible to adequately compare the two settings.

On the other hand, B0/W148 isolates were found at similar rates in *M. tuberculosis* populations in prison and civilian settings in Archangel, North Russia (38, 39), with rates of 21.1% and 16.8% ($P = 0.5$), respectively. This lack of a significant difference is especially apparent in light of the significantly higher rate of the Beijing family as a whole in the local prison versus civilian populations, with rates of 76.3 versus 44.5% ($P = 0.000002$). Likewise, in the central Russian region of Tula (41), B0/W148 constituted similar proportions of the local Beijing genotype populations in the civilian and prison settings, namely, 23.7 and 16.3%, respectively, i.e., the rate was still somewhat higher in prison, but still nonsignificantly ($P = 0.4$). Interestingly, in the earlier Azerbaijan prison study (52), B0/W148 was found at a similar (compared to that for Tula, Russia) rate (21.7%) among Beijing isolates. On the other hand, looking at the rate of B0/W148 prevalence in the total local populations, it was significantly more prevalent in the Tula prison setting (OR [95% confidence interval], 2.99 [1.24, 7.17]). When meta-estimation of the B0/W148 rate in *M. tuberculosis* populations in prison versus civilian settings was performed on the two available locations, Tula and Archangel, the pooled OR (95% confidence interval) under a random-effect model was 1.88 (0.85, 4.17), and the overall effect was nonsignificant ($P = 0.12$). Thus, no marked difference in transmission of B0/W148 versus other Beijing strains was observed in Russian settings, although a slightly higher rate of B0/W148 in prison than in civilian settings should be noted, and further accumulation of this kind of data may eventually clarify this issue.

To sum up this section, there is an indication, but not yet a systematic proof, of the significantly enhanced virulence and increased transmission capacity of the Beijing B0/W148 strain.

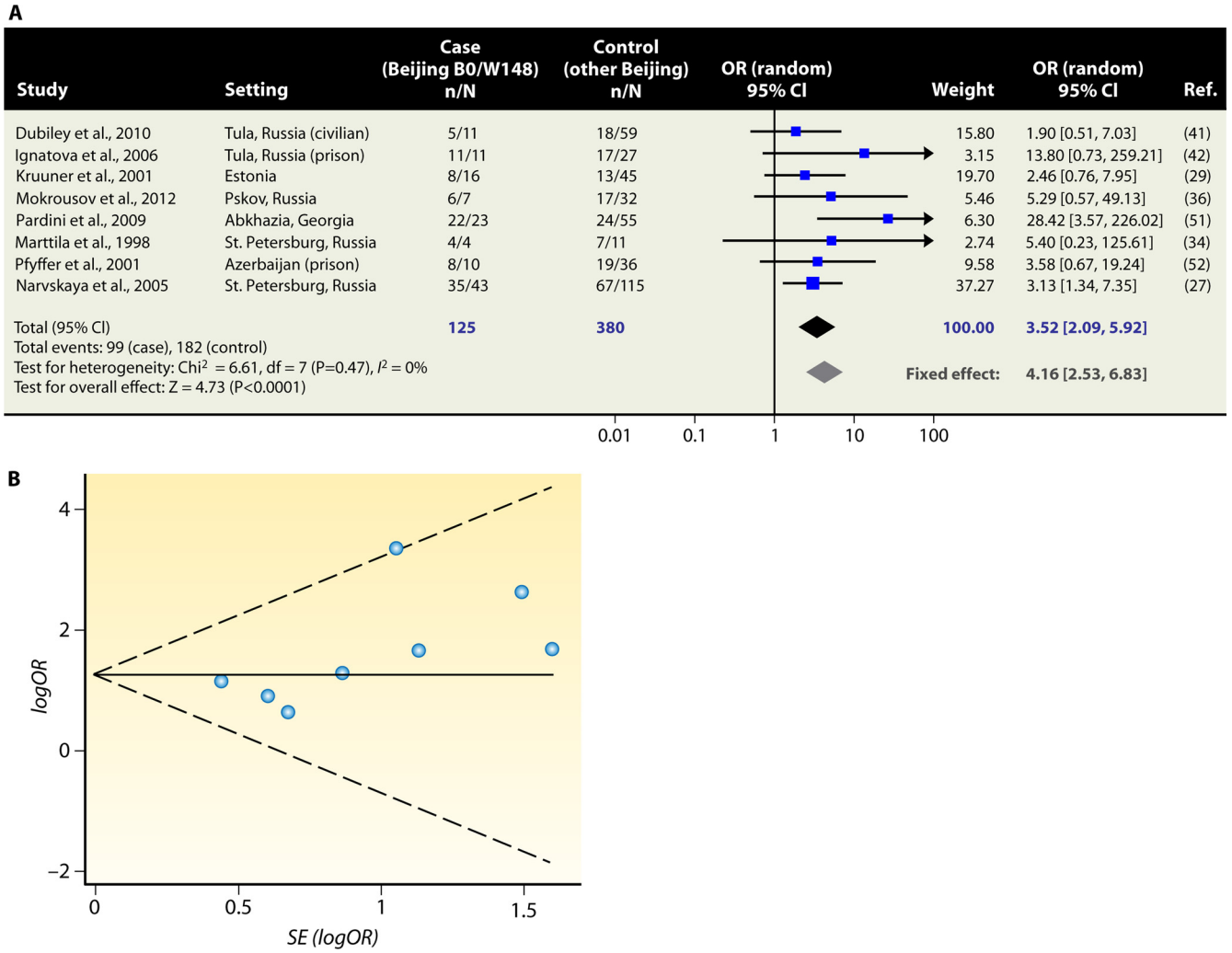

FIG 5 (A) Meta-analysis of the association between *M. tuberculosis* Beijing B0/W148 variant and multidrug resistance. Error bars indicate 95% confidence intervals (95% CI). Solid squares represent each study in the meta-analysis. The solid black diamond represents the pooled OR; the gray diamond shows the fixed-effect model pooled OR (in a test for overall effect, $Z = 5.64$ [$P < 0.0001$]). (B) Begg's funnel plot with pseudo-95% confidence limits. The references in this figure correspond to references 41, 42, 29, 36, 51, 34, 52, and 27.

Drug Resistance

The association with drug resistance appears to be the most available and reliable proxy to assess the extent of danger posed by the circulation of the B0/W148 strain, since the published data are relatively abundant. The first article on Russian strains isolated in the mid-1990s already showed MDR (defined as simultaneous resistance to rifampin and isoniazid, two key anti-TB drugs) to be highly prevalent among B0/W148 isolates (34). B0/W148 isolates constituted 37.2% of drug-resistant Beijing strains in Estonia, while no susceptible B0/W148 isolates were found in that study, which enrolled strains isolated in 1994 (29). More recently, B0/W148 isolates were found to be associated with multidrug resistance in an epidemiologically meaningful subsample of newly diagnosed patients in St. Petersburg, in northwestern Russia ($P < 0.0001$) (26), and the Tula oblast, in central Russia ($P = 0.01$) (41). In Abkhazia, 22 of 23 B0/W148 isolates were MDR, while other Beijing genotype isolates included a notable number of pansusceptible isolates (10 of 55 isolates) (51). In prison settings, this association was even more remarkable: all 11 B0/W148 isolates in Tula and 8 of 10 B0/W148 isolates in Azerbaijan were MDR, in contrast to the more diverse samples of other Beijing genotype isolates, which also included susceptible and monoresistant isolates (42, 52).

A meta-analysis was performed to systematically summarize the available studies and to provide a meta-estimate of the association between the Beijing B0/W148 variant and multidrug resistance (Fig. 5). A control group included other Beijing family strains, since multiple previous studies demonstrated that Beijing family strains in the countries of the former Soviet Union, including Russia, are associated with drug resistance (29, 36, 43). Begg's test provided no evidence of funnel-plot asymmetry ($Z = 1.36$; $P = 0.17$) (Fig. 5B), i.e., there was a lack of publication bias. The eight studies were not significantly heterogeneous but differed in study design, which is why both random- and fixed-effect models were tested. Both showed similar outcomes, and the overall effect, i.e., a stronger association of the B0/W148 strain and multidrug resis-

tance, was significant in both models (Fig. 5A). Meta-analysis was additionally done for case-control comparisons in which B0/W148 (case group) was compared to either all other *M. tuberculosis* strains or non-Beijing genotypes. Under the random-effect model, the overall effect, i.e., increased odds of being MDR for B0/W148, was found to be significant for both kinds of comparisons ($P < 0.0005$), and the pooled ORs (95% confidence intervals) were 6.58 (2.56, 16.92) and 9.97 (2.76, 36.05), respectively.

HUMAN POPULATION CONTEXT

Geographic Diversity and Place of Origin of B0/W148

A precise determination of the place of origin of the B0/W148 strain remains elusive. The location with the highest prevalence rate and highest level of diversity may provide a helpful indication.

The geographical distribution of the Beijing genotype and Beijing B0/W148 strains in different populations is shown in Fig. 2. The prevalence of the Beijing B0/W148 variant varies across Russian regions, but in most cases it is above 10%, being, in particular, 16% in northern European Russia (Archangel) and 19 to 22% in Siberia. Slight decreases are observed in the westward peripheral regions of European Russia (7.9% in Pskov), in Russia's neighbors (7.5% in Estonia, to the northwest, and 7% in Abkhazia/Georgia, to the southwest), and in Moscow. Furthermore, the rate of B0/W148 prevalence sharply decreases in former Soviet Central Asia, being only 3.6% in Kazakhstan and 2.4% in the more distant countries Uzbekistan and Turkmenistan.

As described in Sampling and Methods, the B0/W148 cluster profiles were searched in publications on other areas where the Beijing genotype is endemic, such as East Asia and South Africa, as well as in Russian/ex-Soviet neighbors in Eastern Europe and Central Asia. The B0/W148 variant was not found in East Asian countries marked by endemicity and a high prevalence of the Beijing genotype or in a country with its own particular subset of Beijing strains (South Africa) (see Table S1 in the supplemental material). No or rare isolates of the Beijing family were recovered from permanent residents of Eastern European countries such as Poland, Bulgaria, and Romania (75, 76, 119, 120), and it is reasonable to assume a negligible, if any, presence of the Beijing B0/W148 variant in this part of Europe. None of the above countries received a significant influx of immigrants from the former Soviet Union, and this situation is not unexpected. The Russian (ex-Soviet) emigration has been directed toward the developed world, in regions of western Europe and North America (121). Indeed, the B0/W148 strain has been isolated from Russian immigrants in the United States (28), and while reports from Sweden and Germany described Beijing genotype strains recovered from immigrants of different origins, the B0/W148 strain was isolated only from TB patients coming from the former Soviet Union (30, 80). In Germany, the B0/W148 cluster included mainly patients from countries of the former Soviet Union, first of all Russia. In contrast, other Beijing genotype isolates represented more countries of origin, without a bias toward a single country (30) (see Table S1). In total, the B0/W148 strain was isolated more frequently from Russian immigrants than from other immigrants, though not significantly, apparently due to the small sample size ($P = 0.09$). In further contrasting immigrants coming from Russia and the European part of the former Soviet Union with those from Asian countries of the former Soviet Union, B0/W148 was prevalent among the former (52.9% versus 16.7%; $P = 0.2$). Likewise, a Swedish study of drug-resistant TB (80) described Beijing genotype strains from immigrants of different origins, but the Beijing B0/W148 strain was isolated only from immigrants from the former Soviet Union, especially from those coming from Russia and the European part of the former Soviet Union (see Table S1).

In summary, B0/W148 is specifically prevalent in the European part of the former Soviet Union, and at the same time it is found at high rates in Russian Siberian settings (Novosibirsk and Tuva), making up 19 to 22% of local *M. tuberculosis* populations and 33 to 40% of the Beijing subpopulations. This highlights an intriguing gradient of prevalence of the Beijing B0/W148 variant, with the highest rate in the Siberian part of Russia and somewhat lower rates in the European part of the former Soviet Union, from Estonia in the north to Azerbaijan in the south, and including European Russia. Significantly or nearly significantly higher rates of B0/W148 in total populations in comparably designed studies in Siberian versus European Russian settings should be noted (see Table S1 in the supplemental material), i.e., for Tuva versus Pskov (21.8% versus 7.8%; $P = 0.06$) and Novosibirsk versus Pskov (19.4% versus 7.8%; $P = 0.05$). At the same time, B0/W148 was also more prevalent in Archangel, in northern European Russia, than in Pskov, with rates of 16.8% versus 7.8%, a nearly significant difference ($P = 0.08$).

Furthermore, to estimate the diversity in B0/W148 strains across Russia, I used the published VNTR data on St. Petersburg, in northwestern European Russia (8), and Novosibirsk, West Siberia (44, 45), to build phylogenies of the Beijing genotype and the Beijing B0/W148 variant. These two settings were selected based on the available information on high-resolution VNTR loci typed for these strains. No other studies from other locations that met these criteria were available. As a whole, both trees show a clear divergence of the isolates from these distant Russian locations (Fig. 6), but the nuances in topology are distinctive and intriguing. In the Beijing dendrogram, the subsample from Novosibirsk makes a compact subtree compared to the more diverse sample from St. Petersburg (Fig. 6A); the simplest explanation is the larger sample size of the latter collection. However, and in contrast to the all-Beijing tree (Fig. 6A), the Beijing B0/W148 minimum spanning tree shows not only a divergence of the two settings but also strikingly different topologies of their subtrees (Fig. 6B). One should note that evolution by loss rather than gain of repeats has been suggested as a major mode of evolution of the VNTR loci in *M. tuberculosis*, based on mathematical modeling (122). In this sense, the MST shown in Fig. 6B appears to corroborate this hypothesis and is therefore valid: note the allelic changes on the edges of the tree, which in most cases are reductions of the number of repeats. By VNTR typing, 45 B0/W148 isolates from St. Petersburg were subdivided into 9 types with a single predominant type (star-like phylogeny), thus suggesting a recent/outburst dissemination. In contrast, 14 B0/W148 isolates from Novosibirsk were subdivided into 6 types without a single predominant profile, and presenting a more chain-like phylogeny, thus suggesting a longer period of evolution (Fig. 6B). The Hunter-Gaston indexes (estimations of the VNTR-based diversity) of the St. Petersburg and Novosibirsk B0/W148 samples were 0.43 and 0.79, respectively. Taken together, these findings, i.e., (i) the highest rates of B0/W148 strain prevalence in Novosibirsk and Tuva, (ii) the highest level of diversity of B0/W148 in Novosibirsk, and (iii) the longer evolution of B0/W148 in Novosibirsk, suggest the (West) Siberian

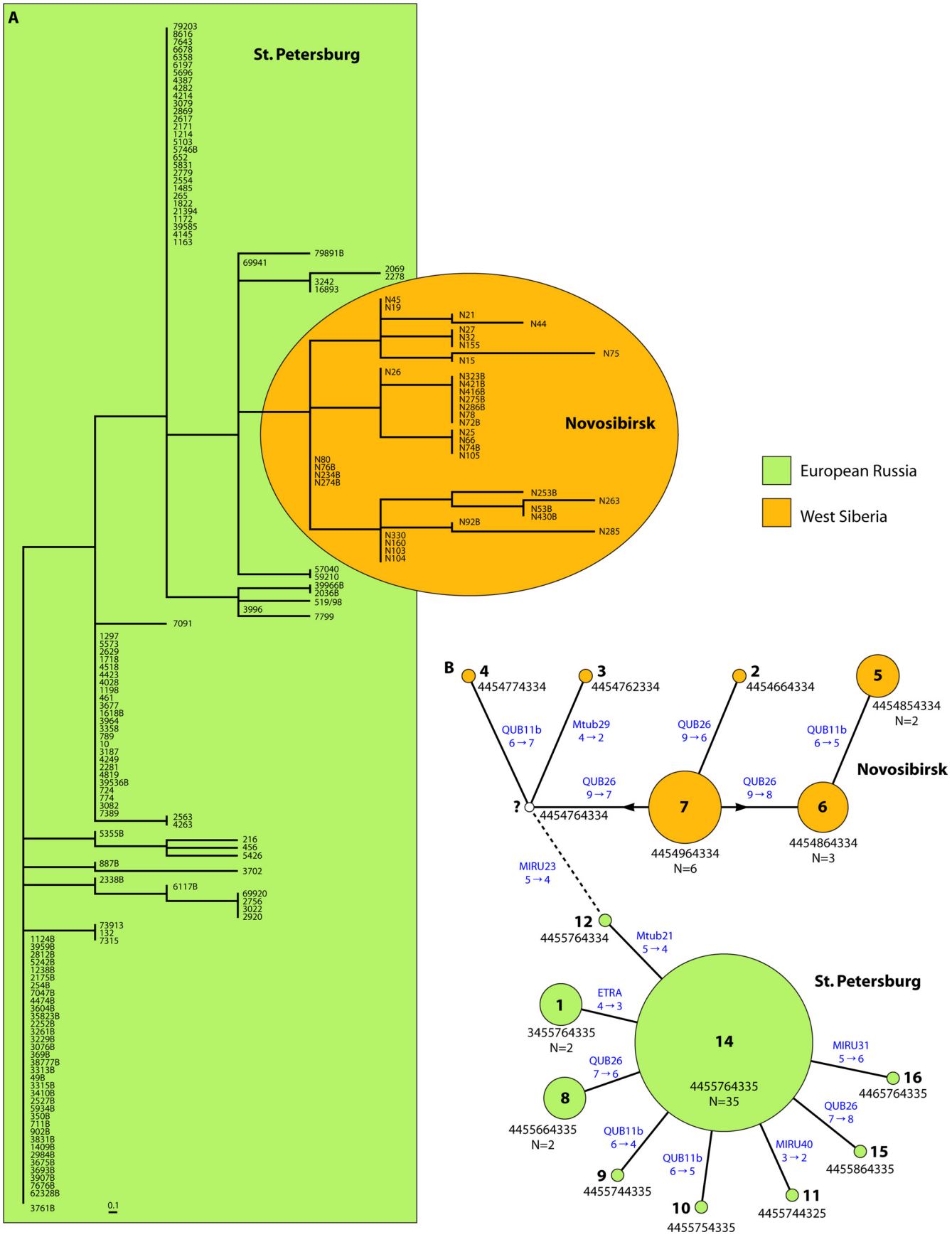

FIG 6 Phylogenetic analysis of 10-VNTR-locus profiles of Beijing strains from Novosibirsk, West Siberia, and St. Petersburg, northwestern European Russia. (A) Neighbor-joining tree for Beijing genotype strains. (B) Minimum spanning tree for Beijing B0/W148 strains. Type numbers are for convenience only. Digital VNTR profiles show copy numbers for the following loci: ETR-A, ETR-C, MIRU31, MIRU23, QUB26, QUB11b, Mtub29, Mtub39, MIRU40, and Mtub21. Each arm depicts one locus change.

area of Russia to be a hypothetical area of origin of the B0/W148 variant.

Timing of Origin and Primary Dispersal of B0/W148

Clearly, B0/W148 did not originate in the two most recent decades: in such a case, it would not be found in Tuva, which was marked with not an influx but a sharp outflow of ethnic Russians, exactly since the 1990s (123). Furthermore, in the early/mid-1990s, B0/W148 isolates had already been described from across the former Soviet Union, in locations as distant as northwestern Russia, Estonia, and Azerbaijan. Sinkov et al. (124, 125) recently proposed an intriguing hypothesis about Gulag-driven importation of the Beijing genotype into, and its primary dispersal across, the former Soviet Union, beginning in the 1930s. Although its first penetration into Russia is open to question (see the work of Mokrousov et al. [17] for an earlier timing of the first penetration of the Beijing genotype into northern Eurasia), the Beijing genotype was indeed most likely disseminated throughout the country due to a massive population mixing in the Soviet time of industrialization, urbanization, and concentration camps of the Gulag penitentiary system. However, could this also be the case for the B0/W148 variant? The Archangel area in North Russia and the studied Siberian settings (Novosibirsk, Tuva, and the Mariinsk prison in the Kemerovo oblast [see above]) represent the past (Gulag) and current penitentiary systems, seemingly reemphasizing an accelerated transmission of the Beijing B0/W148 strain under prison/concentration camp conditions. However, the Gulag system covered almost all of Russia, not only the northern and Siberian areas but also Kazakhstan and central Asia (126). The Gulag system began shrinking in 1954 and was closed in 1960. In this view, the Gulag system could not be the source of dissemination of the B0/W148 strain throughout the country. Furthermore, the B0/W148 variant could not have started its emergence before 1960. In such a case, it would have spread across all of the former Soviet Union, to both its European and Asian parts, similar to the spread of the Beijing genotype as a whole. A speculative explanation is that B0/W148 originated in Siberia before 1960, but not inside the Gulag system, and remained confined to its unknown niche/host population until some social event triggered its emanation beyond Siberia.

A closer look at the data on Soviet demographics reveals two landmark events that involved mass human movement in the former Soviet Union in the 1950s to 1980s: (i) a virgin land campaign (*tselina*) from 1955 to 1960 and (ii) population outflow from Siberia, especially West Siberia, in the 1960s to 1980s (127–129) (Fig. 5).

A mass migration from European Russia (but not Siberia) to Kazakhstan from 1955 to 1960, within the frame of the virgin land campaign, dramatically decreased the proportion of Kazakhs within the population, from 57% in 1926 to 30% in 1959. Furthermore, after the end of the campaign, 6 million ethnic Russians and Ukrainians stayed in Kazakhstan (130; http://en.wikipedia.org/wiki/Virgin_Lands_Campaign), and hence their interaction with the local population continued in the coming decades. If B0/W148 had been circulating in European Russia/Ukraine before 1960, it would have been brought to Kazakhstan in 1955 to 1960 and would have spread there. Apparently, this was not the case, since its rate in Kazakhstan is only 3.6%. It should be kept in mind that the mass virgin land campaign of 1954 to 1960 targeted not only Kazakhstan but also less inhabited and less developed areas in Ural and West Siberia (131). Consequently, as it was not brought to Kazakhstan from European Russia during the virgin land campaign, B0/W148 could not have been brought to Siberia from European Russia either. To sum up, B0/W148 isolates did not circulate across European Russia before 1960.

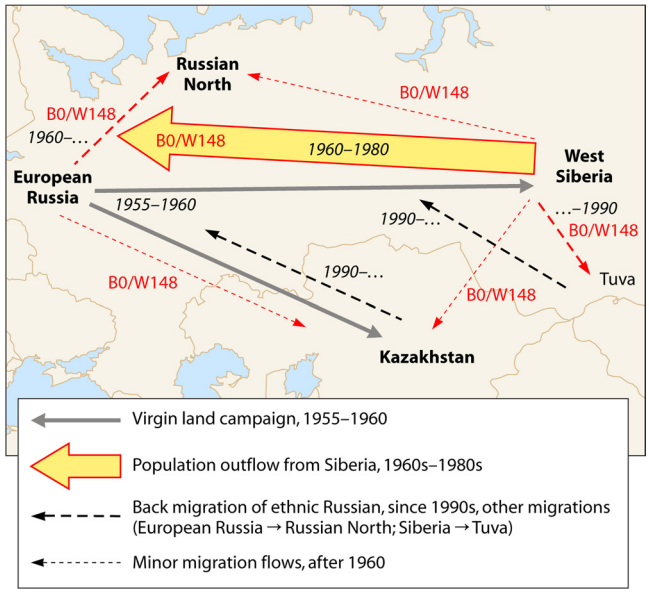

FIG 7 Direction and timing of major flows of human migration associated with origin and dispersal of the Beijing B0/W148 strain. The thickness of arrows roughly reflects the volume of migration. Red arrows indicate the hypothesized dissemination of the B0/W148 strain.

A remarkable population outflow from Siberia and the Far East to the central and northwestern regions of Russia was another, and opposite (with respect to the virgin land campaign), process that started in the 1960s. Eight hundred thousand persons migrated from West Siberia to European Russia from 1961 to 1970 (127–129, 132). I speculate that this may have been the first dispersal of the B0/W148 variant beyond its hypothetical area of origin, West Siberia (Fig. 7).

The pair of St. Petersburg and Novosibirsk represents both geographically and phylogenetically (in terms of B0/W148 frequency and diversity) distant regions. Both are also areas with high levels of population density and urban development. In contrast, the much less populated and geographically marginal sites of Archangel, North Russia, and Tuva, West Siberia, make another and peculiar pair that deserves no less attention, since it permits a view of the manifestation of the Beijing B0/W148 strain in another socioecological context.

The Beijing B0/W148 cluster is found at a high rate in Tuva. Historically, Tuva (formerly known as Tannu Uriankhai) was influenced by Mongolians and, to some extent, the Chinese, but in both cases this interaction was limited to political relations, with little human exchange, and hence little probability of pathogen exchange. In contrast, Russian colonists settled there only beginning at the end of the 19th century and, furthermore, settled in the sparsely populated northern and central parts; hence, interpopulation transmission of infectious agents was less probable, at least in the first decades (133). Accordingly, B0/W148, even if it existed at that time, would not have been spread by Russians in Tuva.

Tannu Uriankhai was definitely included in the Soviet Union in 1944. The Russian population there increased from 20% in 1918 to 1940 to 36 to 40% in 1960 to 1980 (123, 133). However, the population of ethnic Russians in Tuva has been reduced sharply, to 16%, in the last 2 decades; hence, the B0/W148 strain should have penetrated to Tuva prior to the 1990s. Indeed, the Russian population increased in Tuva in the 1960s to 1980s, up to 40%, and this may have correlated with the first spread of the B0/W148 strain in this region.

Two thousand miles away from Tuva, the Archangel province is located in the Russian North, a specific area of the White Sea and Barents Sea region that is still in the limits of European Russia but marked by a severe climate and a low population density. By all means, Archangel cannot be placed within Siberia. But unlike the case in other parts of European Russia (and the former Soviet Union), the rate of the B0/W148 strain is high in the civilian population of Archangel, and nearly significantly higher than in Pskov, the westernmost region of mainland (European) Russia, located at the Russian-Latvian border (Fig. 2). To explain this situation, the following, sometimes opposing and complex facts may be considered. First, a moderate but permanent population outflow from North Russia has taken place since 1990 (134); hence, B0/W148 must have been brought to this region before 1990. Second, the population in Archangel almost doubled over a span of 30 years, from 256,000 in 1959 to 415,000 in 1989 (http://fr.wikipedia.org/wiki/Arkhangelsk), which could have been due at least partly to incoming migration from other Russian regions (129). Third, a rotation work system has been practiced in North Russia, and temporary workers could, in principle, come from either Siberia or European Russia. Migration from Siberia would seem to be a less significant factor, as people in this area tend to change/soften their climate conditions rather than move to a similar hard climate. Hence, rotation migrants more likely moved to Archangel from European Russia, and furthermore, the newcomers in the harsh climate and hard living conditions of the North may have experienced stress leading to increased morbidity (135). Fourth, the low genetic diversity of the B0/W148 cluster in Archangel (38), reflecting its recent evolution *in situ*, thus readily corroborates the hypothesis about a mainly secondary, European source of these strains in the Russian North. To summarize, the B0/W148 strain was likely brought to Archangel not from its primary focus, Siberia, but rather from European Russia following the secondary wave of its dispersal across the country (Fig. 7). Reactivated through climate conditions, the B0/W148 clone has ultimately reached a high rate there, higher than in its possible source area, central Russia.

To sum up, based on the historical, demographic, and ecological knowledge and molecular data on the diversity and frequency of the Beijing B0/W148 strain across the former Soviet Union, most parsimoniously, this *M. tuberculosis* variant likely originated in Siberia, and before 1960. Primary dispersal of B0/W148 outside Siberia started after 1960, driven by the population outflow from Siberia to European Russia/former Soviet Union but not to former Soviet Central Asia. This hypothetical scenario is summarized in Fig. 7.

Driving Forces behind Emergence and Spread of B0/W148

The above reasoning links particular human migration flows to the dissemination of B0/W148 in Russia since the 1960s. The next question is as follows: why has its spread become so dramatically successful (as exemplified by the star-like phylogeny in Fig. 4)? What event, along with human migration, triggered the emergence in and wide propagation of this strain from Siberia across the country and within new areas in European Russia? I hypothesize that the advent of modern drugs for anti-TB treatment was such a catalyzing circumstance. As a brief reminder, the relatively recent milestones within the course of the history of anti-TB chemotherapy are, in particular, the introduction of ethambutol in the 1960s and the recognition of the crucial role of isoniazid and rifampin in the 1970s and of pyrazinamide in the 1980s (136). All of these events correspond to the above-hypothesized timeline of the emergence and dispersal of B0/W148 beyond Siberia. While proof of the enhanced virulence of the B0/W148 strain is unsystematic and controversial, it is remarkable that the above meta-analysis demonstrated a strong association of B0/W148 with multidrug resistance. In this sense, the primary spread of the B0/W148 strain outside Siberia since the 1960s was not just coincidence but most likely was catalyzed and fueled by a finalization of the modern anti-TB regimen and wide use of the key first-line anti-TB drugs.

In contrast, a link between spread of the B0/W148 strain and its capacity to evade the protective effect of BCG vaccination is much less apparent, although not completely impossible. Regarding the Beijing family as a whole, it has previously been hypothesized that these strains have acquired mechanisms to circumvent BCG-induced immunity; nonetheless, the association of the emergence of the Beijing genotype and BCG vaccination remains inconclusive (14). It should be noted that BCG vaccination was widely implemented in the Soviet Union in both rural and urban areas beginning in the 1930s. On the other hand, BCG vaccination of newborns became mandatory in 1952 (137), thus coinciding with the hypothesized emergence of the B0/W148 variant in Siberia and its primary dispersal to European Russia since the 1960s.

CONCLUSIONS AND PERSPECTIVES

To sum up, in this review, placing clinical, epidemiological, and molecular data in a broad historical, demographic, and ecological context, I put forward two interdependent hypotheses. First, the Beijing B0/W148 strain likely originated in Siberia, and its primary dispersal was driven by a massive population outflow from Siberia to European Russia in the 1960s to 1980s. Second, a historically recent emergence and dissemination of B0/W148 were triggered by the advent and wide use of modern anti-TB drugs and were due to the remarkable capacity of this variant to acquire drug resistance. In contrast, there is some indication, but not yet a substantial and systematic proof, of enhanced virulence of this strain. Nonetheless, the star-like topology of the high-resolution VNTR network does suggest a historically recent and/or outburst dissemination of this clonal cluster in its new, secondary focus, northwestern Russia, thus supporting the designation of B0/W148 as a successful clone. Furthermore, the historical spread of the Beijing B0/W148 variant across Russia in the last 50 years is currently evolving toward a global propagation of this strain by Russian emigrants and its circulation in respective immigrant communities in Western Europe and the United States. This situation should be taken into consideration by national TB control programs, especially in view of the remarkable propensity of B0/W148 to develop multidrug resistance. In this regard, a recently described, simple PCR-based method to rapidly detect this strain

(33) may become a useful complement to the currently used molecular methods of *M. tuberculosis* diagnostics and typing.

As demonstrated in this review, in spite of the common perception about the omnipresence of B0/W148 across all post-Soviet space, a closer look at its geographic distribution reveals a peculiar clinal gradient. Its rate peaks in Siberian Russia and, to a lesser extent, in the European part of the former Soviet Union. In contrast, the rate of B0/W148 is sharply decreased in the Asian part of the former Soviet Union. One explanation is that an ordinary human exchange *per se* is not enough to introduce a new tuberculosis strain into an indigenous population, even in the present time of urbanization and overcrowding. There may be a kind of human resistance developed in the local population through its coexistence with historically established clones, counteracting an invasion of new, "alien" strains. For example, the Beijing family as a whole is present at high rates in Latvia and Estonia, most likely as a result of the large influx (demic diffusion) of Russian migrants from 1944 to 1991. In contrast, the Beijing genotype is not found in autochthonous populations in Eastern Europe: while the Eastern European countries were part of the Warsaw Treaty, they did remain independent, also keeping a singularity of their microbial pools. Similar to the Russian/European history of the Beijing family, dissemination of the Beijing B0/W148 strain apparently required a massive human movement, such as the population outflow from Siberia to the European part of the former Soviet Union. In the absence of such a significant human flow from Siberia to former Soviet Central Asia, the B0/W148 strain is still found at negligible rates in Kazakhstan, Kyrgyzstan, Uzbekistan, and Turkmenistan. In this regard, a study of human candidate genes with relationships to host susceptibility to tuberculosis caused by Beijing B0/W148 compared to other *M. tuberculosis* strains in different ethnic groups is warranted. A number of previous studies demonstrated a coadaptive interaction of certain human alleles and endemic *M. tuberculosis* genotypes, manifested as different clinical outcomes in different hosts infected with the same strain (138–142). For example, a lethal effect of the Beijing genotype that is exerted more frequently on *CD209* −336G male carriers in Slavic and East Asian populations has been hypothesized (142).

The especially high rate of multidrug resistance among actively circulating Beijing B0/W148 isolates suggests that certain compensatory genome mutations may have been retained through second-order selection mechanisms that permit a rapid acquisition of drug resistance without reducing the pathogenic capacity of a strain. To study the biological fitness of the B0/W148 strain, a far-from-exhaustive list of target genes includes 3R genes (recombination, replication, and repair genes) (143), secondary members of the drug resistance-associated groups of genes (such as *rpoA* and *rpoC* [144] and the *embCAB* operon), and the virulence-related *eis*, *mce*, and *esx* gene families. Other studies of relatively simple design may be envisaged to elucidate the fitness-related features of the B0/W148 strain, e.g., studies of the biological cost of drug resistance mutations through evaluations of the growth rate and mixed-infection model studies to see if this strain can outcompete other strains.

Last but not least, efforts to fight TB also include the development of new vaccines and novel anti-TB drugs. Both should undoubtedly show their efficacy against the predominant strains of tubercle bacilli in order to be adopted for therapeutic use in the next decade. In this sense, Beijing B0/W148 is an important candidate for inclusion in the panel of successful *M. tuberculosis* strains that may be ideal candidates for future studies on vaccine development and for assessing the therapeutic efficacy of new drugs.

Future studies on more isolates from new locations and, most importantly, new time points (i.e., focused on archival specimens) will increase the density of data and the resolution of analysis, test the proposed hypothesis about the place and time of origin of the *M. tuberculosis* Beijing B0/W148 strain, and shed more light on the pathogenomics-related features lying behind its emergence and spread in the human population.

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