Department of Medicine, University of California San Francisco, San Francisco, CA 94121, USA (IR-B); Mathematical Modelling of Infectious Diseases Unit, Institut Pasteur, Paris, France (HS); Centre National de la Recherche Scientifique, URA3012, Paris, France (HS); Centre of Bioinformatics, Biostatistics and Integrative Biology, Institut Pasteur, Paris, France (HS); Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA (HS); Department of Biology, University of Florida, Gainesville, FL, USA (HS, DATC); and Emerging Pathogens Institute, University of Florida, Gainesville, FL, USA (DATC)

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Multidrug-resistant tuberculosis outbreak in South Africa

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We note with concern the Article by Ndivhuho Makhado and colleagues[1] that described the high frequency of Ile491Phe mutations in the *rpoB* gene among a small subset of highly selected isolates of *Mycobacterium tuberculosis* from South Africa. In a letter to *The Lancet Infectious Diseases*,[2] we previously refuted the likelihood of this mutation being widespread; therefore, we are disappointed that the mutation is now being claimed as the cause of an outbreak of multidrug-resistant tuberculosis and used to challenge current diagnostic methodologies that are the bedrock of national tuberculosis programmes.

A population-representative tuberculosis drug-resistance survey using whole-genome sequencing,[3] done at the same time as the study by Makhado and colleagues,[1] showed the true prevalence of the Ile491Phe mutation to be less than 0·1% (one in 1535) among patients with tuberculosis from two provinces neighbouring eSwatini.[3] The single isolate was rifampicin monoresistant in both phenotype and genotype. Furthermore, none of the 140 isoniazid-monoresistant tuberculosis strains with available sequencing data from the survey, including representation from North West and Mpumalanga provinces, had the mutation.[4] By contrast, Makhado and colleagues[1] used convenience sampling of available culture isolates from a single laboratory serving a small area, and the study was, thus, unsuitable for prevalence estimation. The starting point was culture isolates, a practice that is routinely indicated by the national algorithm only when the results of initial Xpert MTB/RIF testing are negative, or when treatment has failed.[5] This selection bias towards drug-resistant isolates is evidenced by the unusually low treatment success rates for patients with and without the mutation. Therefore, Makhado and colleagues' assertion that their findings are generalisable and that the mutation is responsible for a "substantial number of MDR tuberculosis cases" in South Africa[1] is unfounded.

The study[1] had a number of methodological flaws, including the inappropriate description of the findings as evidence of an outbreak; the absence of a description of duplicate sample management; and the lack of adequate explanations for the sample size selection of 277 of the 1823 isoniazid-resistant rifampicin-sensitive strains, or for whole-genome sequencing being done on only 14 of 37 samples identified by Sanger sequencing as harbouring Ile491Phe. Furthermore, epidemiological data pertaining to patients from whom strains originated, including the exact place of residence, country of origin (eg, eSwatini), and occupation, were not provided. It is not possible, therefore, to ascertain the presence of clustering, nor epidemiological linkage—a necessary criterion in declaring an outbreak. On the basis of the evidence provided, all that can be said is that these isolates are genotypically related, which is expected in endemic settings, but recent transmission cannot be inferred.[6,7] It is also unusual to find isolates with no differences in single-nucleotide polymorphisms when transmission networks span several years: a situation that could be explained by laboratory cross-contamination. The hypothesis that these cases are associated with bedaquiline introduction is also not justified because previous treatment history and contact history are not provided. Furthermore, the geographical location of these cases was far from the initial treatment site for the bedaquiline clinical access programme in the province.[8]

Makhado and colleagues recommend that South Africa adopt an assay used in their study, but three of the authors have declared commercial interests in the company that manufactures it. Although no test can be perfect, the WHO-endorsed technologies in use (GenoType MTBDRplus and Xpert MTB/RIF) are validated, detecting at least 95% of cases of rifampicin resistance.[9] Furthermore, the current algorithm in South Africa requires sputum culture and susceptibility testing when patients fail treatment, and whole-genome sequencing is available and used by the reference laboratory in appropriate circumstances. Until meaningful data become available, we believe this approach is appropriate to safeguard current treatment regimens against development of rifampicin resistance.

We declare no competing interests.

*Nazir A Ismail, Kerrigan McCarthy, Francesca Conradie, Wendy Stevens, Norbert Ndjeka
naziri@nicd.ac.za

Centre for Tuberculosis (NAI) and Division of Public Health Surveillance and Response (KM), National Institute for Communicable Diseases, Johannesburg, South Africa; National Clinical Advisory Committee and University of Witwatersrand, Johannesburg, South Africa (FC); National Priority Programmes, National Health Laboratory Service, Johannesburg, South Africa (WS); and MDR-TB Directorate, National Department of Health, Pretoria, South Africa (NN)

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Authors' reply

We thank Nazir Ismail and colleagues for sharing unpublished findings from genomic data from the South Africa tuberculosis drug-resistance survey.[1] Unlike the objective of this survey, our goal was to specifically determine whether *rpoB* Ile491Phe-bearing *Mycobacterium tuberculosis* isolates obtained from patients with multidrug-resistant (MDR) tuberculosis were misclassified as isoniazid-monoresistant by standard diagnostic algorithms, in a centre receiving samples from four South African provinces.

Samples from all patients referred to the National Health Laboratory Service–Dr George Mukhari Tertiary Laboratory over the study period were cultured, irrespective of Xpert MTB/RIF results or treatment history. We screened isolates randomly selected from 1823 isoniazid-monoresistant strains among 37 644 *M tuberculosis*-positive cultures using four techniques to detect *rpoB* Ile491Phe.

Most of the Ile491Phe mutants were found to have unique individual (albeit closely related) single-nucleotide polymorphism (SNP) profiles by deep sequencing or whole-genome sequencing, excluding duplicates or laboratory contamination. Four isolates had indistinguishable genome sequences, commonly seen in transmission networks spanning several years.[2,3] These genomically identical isolates, like others separated only by a single SNP, were obtained within a 2-year period from different local clinics located within 20 km of one another. Such conjunction of spatiotemporal clustering and close genomic relatedness, with a rare compensatory mutation (*rpoC* Glu1033Ala) and other shared drug-resistance associated mutations, is strongly supportive of an outbreak.[2,3] Our results delineate the minimal outbreak extent, and we therefore suggested that further studies were warranted to assess the prevalence and spread of this mutation outside of the study population.

Although we agree with Ismail and colleagues, as well as Variava and Martinson,[4] that the association of the detected *Rv0678* mutations with bedaquiline resistance awaits formal demonstration, the observed independent emergences of different *Rv0678* mutations in groups of otherwise indistinguishable genomes are very unlikely to be due to simple genetic drift. Other *Rv0678* mutations are associated with resistance to bedaquiline or clofazimine.[5,6] Our temporal reconstruction suggests that such mutations arose around the year 2015, 2 years after the start of the bedaquiline clinical access programme, compatible with a link with bedaquiline use.

It is unfortunate that not all resistance mutations in *M tuberculosis* are picked up by rapid tests in use, and the *rpoB* Ile491Phe mutation is not the only resistance mutation missed. Moreover, such undetected mutants are not unique to South Africa.[7] Similarly to treatment regimens, diagnostic tests can drive amplification and spread of resistance. As concluded by Variava and Martinson,[4] better tests are needed to cope with the global problem of MDR tuberculosis.

PS is a consultant for Genoscreen. All other authors declare no competing interests.

*Ndivhuho A Makhado, Maphoshane Nchabeleng, Philip Supply, Bouke C de Jong, *Emmanuel André
emmanuel.andre@uzleuven.be

Department of Medical Microbiology, National Health Laboratory Service–Dr George Mukhari Tertiary Laboratory, Pretoria, South Africa (NAM, MN); Department of Microbiological Pathology, Sefako Makgatho Health Sciences University, Pretoria, South Africa (NAM, MN); Mycobacteriology Unit, Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium (NAM); Global Health Institute, University of Antwerp, Belgium (NAM); Centre National de la Recherche Scientifique, Lille, France (PS); Institut National de la Santé et de la Recherche Médicale, Lille, France (PS); Centre Hospitalier Universitaire de Lille, Lille, France (PS); University of Lille, Lille, France (PS); U1019, UMR 8204, Centre for Infection and Immunity of Lille, Institut Pasteur de Lille, Lille, France (PS); Mycobacteriology Unit, Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium (BCdJ); and Laboratory of Clinical Bacteriology and Mycology, Department of Microbiology and Immunology, KU Leuven, Leuven 3000, Belgium (EA)

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