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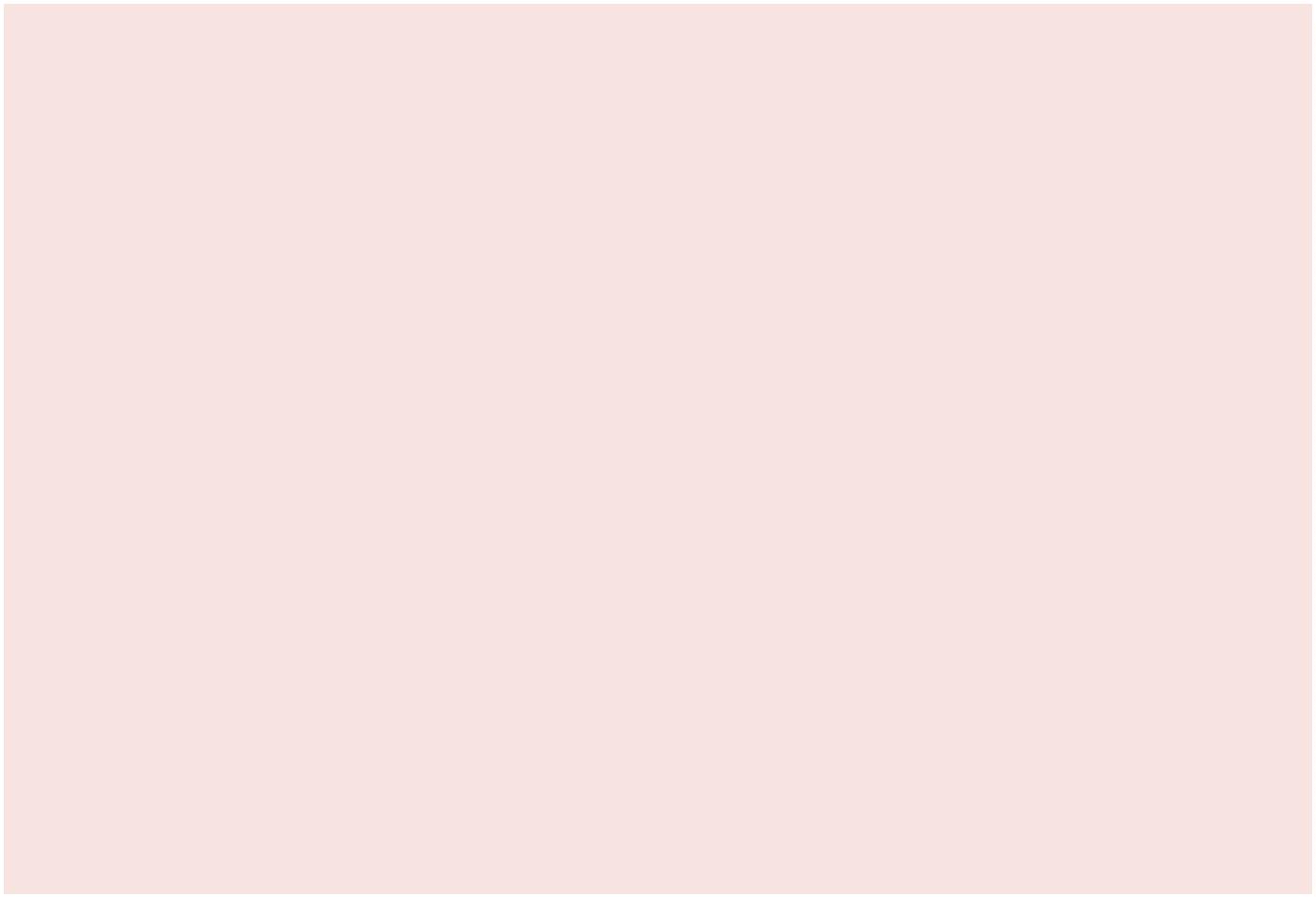
The 2021 WHO catalogue of Mycobacterium tuberculosis complex mutations associated with drug resistance:a genotypic analysis

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| Summary  Background Molecular diagnostics are considered the most promising route to achievement of rapid, universal drug susceptibility testing for Mycobacterium tuberculosis complex (MTBC). We aimed to generate a WHO-endorsed catalogue of mutations to serve as a global standard for interpreting molecular information for drug resistance prediction.  Methods In this systematic analysis, we used a candidate gene approach to identify mutations associated with resistance or consistent with susceptibility for 13 WHO-endorsed antituberculosis drugs. We collected existing worldwide MTBC whole-genome sequencing data and phenotypic data from academic groups and consortia, reference laboratories, public health organisations, and published literature. We categorised phenotypes as follows: methods and critical concentrations currently endorsed by WHO (category 1); critical concentrations previously endorsed by WHO for those methods (category 2); methods or critical concentrations not currently endorsed by WHO (category 3). For each mutation, we used a contingency table of binary phenotypes and presence or absence of the mutation to compute positive predictive value, and we used Fisher’s exact tests to generate odds ratios and Benjamini-Hochberg corrected p values. Mutations were graded as associated with resistance if present in at least ﬁve isolates, if the odds ratio was more than 1 with a statistically signiﬁcant corrected p value, and if the lower bound of the 95% CI on the positive predictive value for phenotypic resistance was greater than 25%. A series of expert rules were applied for ﬁnal conﬁdence grading of each mutation. |  | Lancet Microbe 2022;  3: e265–73  Published Online  March 8, 2022  https://doi.org/10.1016/ S2666-5247(21)00301-3  \*Contributed equally †Contributed equally  ‡Member of the CRyPTIC Consortium http://www. crypticproject.org.  §Member of the Seq&Treat Consortium https://www.finddx. org/at-risk-populations/seq- treat/  Other members of the CRyPTIC Consortium and of the Seq&Treat Consortium are listed in appendix 1 |
| Findings We analysed 41137 MTBC isolates with phenotypic and whole-genome sequencing data from 45 countries. 38215 MTBC isolates passed quality control steps and were included in the ﬁnal analysis. 15 667 associations were computed for 13211 unique mutations linked to one or more drugs. 1149 (7·3%) of 15667 mutations were classiﬁed as associated with phenotypic resistance and 107 (0·7%) were deemed consistent with susceptibility. For rifampicin, isoniazid, ethambutol, ﬂuoroquinolones, and streptomycin, the mutations’ pooled sensitivity was more than 80%. Speciﬁcity was over 95% for all drugs except ethionamide (91·4%), moxiﬂoxacin (91·6%) and ethambutol (93·3%). Only two resistance mutations were identiﬁed for bedaquiline, delamanid, clofazimine, and linezolid as prevalence of phenotypic resistance was low for these drugs.  Interpretation We present the ﬁrst WHO-endorsed catalogue of molecular targets for MTBC drug susceptibility testing, which is intended to provide a global standard for resistance interpretation. The existence of this catalogue should encourage the implementation of molecular diagnostics by national tuberculosis programmes.  Funding Unitaid, Wellcome Trust, UK Medical Research Council, and Bill and Melinda Gates Foundation.  Copyright © 2022 The Author(s). Published by Elsevier Ltd. This is an Open Access article under the CC BY 4.0 license.   |  |  |  | | --- | --- | --- | | Introduction  In 2020, an estimated 1·4 million fewer people than in 2019 received treatment for tuberculosis because of disruptions related to the COVID-19 pandemic.1 The estimated 1·9 million deaths from tuberculosis in 2020, 500000 more than in the previous year, will set the world back to levels of mortality not seen since 2010.1,2 The diagnosis and appropriate treatment of patients with rifampicin resistance was already a challenge before the pandemic, with less than half of patients beneﬁting from access.2 The availability of several new treatment options |  | and strategies is progress, but getting the right drugs to the right patients in time to inﬂuence outcomes positively is essential and requires access to rapid and accurate diagnostics that meet the emerging needs.3,4  WHO set an important but challenging target on universal drug susceptibility testing (DST), which includes testing for the new and repurposed drugs in the WHO revised deﬁnition of extensively drug-resistant tuberculosis.5 Phenotypic DST for Mycobacterium tuberculosis complex (MTBC), although still the reference standard for most drugs, can take over a month to | |  | Nuffield Department of Medicine, University of Oxford, Oxford, UK (T M Walker DPhil,  P W Fowler PhD, J Knaggs BSc,  M Hunt PhD,  Prof T E A Peto FRCP,  Prof A S Walker PhD,  Prof D W Crook FRCPath); Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam (T M Walker); IRCCS San Raffaele Scientific Institute, Milano, Italy  (P Miotto PhD, D M Cirillo PhD); Department of Genetics, University of Cambridge, Cambridge, UK (C U Köser PhD); European Bioinformatics Institute, Hinxton, UK  (J Knaggs, Z Iqbal DPhil, M Hunt); Imperial College  London, London, UK  (L Chindelevitch PhD); Havard Medical School, Boston, MA, USA (M R Farhat MD);  Biomedicine Institute of Valencia IBV-CSIC, Valencia, Spain (I Comas PhD); CIBER Epidemiology and Public Health, Madrid, Spain  (I Comas); Centers for Disease Control and Prevention, Atlanta, GA, USA (J Posey PhD); National Institute for  Communicable Diseases, |

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| Johannesburg, South Africa  (S V Omar PhD); National Institutes for Health Research  Oxford Biomedical Research  Centre, Oxford, UK  (Prof T E A Peto, Prof A S Walker, Prof D W Crook); FIND, Geneva,  Switzerland (A Suresh MSc,  S Uplekar PhD, S Laurent PhD,  Prof T C Rodwell MD,  R E Colman PhD); Global  Tuberculosis Programme, WHO, Geneva, Switzerland  (C-M Nathanson PhD,  M Zignol MD,  N Ismail FRCPath [SA]); Division of Pulmonary, Critical Care and  Sleep Medicine, University of  California, San Diego, CA, USA  (Prof T C Rodwell)  Correspondence to: Dr Timothy M Walker, Nuffield  Department of Medicine,  University of Oxford,  Oxford, OX3 9DU, UK  timothy.walker@ndm.ox.ac.uk  or  Prof Timothy C Rodwell, Division  of Pulmonary, Critical Care and  Sleep Medicine, University of  California, San Diego, CA  92093, USA  trodwell@health.ucsd.edu | | |  | Research in context  Evidence before this study  We searched PubMed using the search terms “tuberculosis”, “mutation” and “catalogue” (interchanged with “database”)  for primary research articles in English from database inception to Feb 19, 2022. We identiﬁed publications that used catalogues for drug resistance prediction tools, as well as a catalogue of phylogenetic mutations that was used to inform our work. Among the databases we identiﬁed were ones that focused on as few as seven genes, and others that were now outdated. We identiﬁed the systematic review of the literature by ReSeqTB, an early precursor of our work, and a second systematic review covering only new and repurposed drugs. There has to date been no WHO endorsed standard of Mycobacterium tuberculosis complex (MTBC) mutation and interpretation for national tuberculosis programmes or industry to refer to in the design of their services or products.  Added value of this study  The diverse nature of the data accumulated over the past  decades using diﬀerent technologies and diﬀerent platforms has made comparability challenging. Our study leveraged established knowledge to deﬁne a set of candidate genes for each drug and sought to assemble as large a dataset as possible, from as many countries as possible, to do a new analysis of all the available whole-genome sequencing data and associated phenotypes. |  | The result is the ﬁrst WHO endorsed catalogue of mutations associated with drug resistance and consistent with drug susceptibility (published by WHO in 2021) that constitutes  an international reference point for national tuberculosis programmes and assay manufacturers. This analysis reﬂects the data that generated the catalogue, providing a summary of the ﬁndings and an overview of what has been achieved so far, and where future eﬀorts should be invested to improve molecular diagnostics, and thereby also patient care.  Implications of all the available evidence  The WHO catalogue is a deliberately conservative eﬀort and is not exhaustive. The weight of evidence supporting each mutation in the catalogue should, however, provide conﬁdence to the tuberculosis community. Great accuracy has been achieved when predicting drug resistance in the past, but not every mutation in previous catalogues has been as well supported as is the case here. Having a WHO endorsed mutation list should support the ﬁeld of molecular drug susceptibility testing in MTBC by providing all countries a template for the interpretation of their data, and thereby encouraging adoption of these technologies. WHO plans to update the catalogue on a regular basis and, as more data are generated as a consequence, these will feed into future analyses and hopefully initiate a virtuous circle. |
|  | See Online for appendix 1 |  | complete and requires expensive, complex laboratory capacity. For many countries these challenges remain prohibitive. Genotypic approaches to susceptibility testing can be rapid, accurate, automated, and cost-eﬀective.6 However, no WHO-endorsed catalogue of mutations for the interpretation of genotypic DST results is available.  The combination of rapid and accurate diagnostic tools and supportive WHO policies can have signiﬁcant global impact.2 WHO previously published a guide to MTBC next-generation sequencing data interpretation, adopting the ﬁndings from a previous systematic review of the literature.7,8 However, that review did not cover new or repurposed drugs and relied on Sanger sequencing results, which meant that the genomic regions interrogated were inconsistent, increasing the chance of false associations. Gaps in knowledge thus remain. We did a systematic analysis of a large, globally diverse set of isolates using whole-genome sequencing (WGS) data and accompanying DST to generate more knowledge and create the ﬁrst WHO- endorsed catalogue of mutations for 13 antituberculosis drugs.9 | |  | accepted whether locally representative or enriched for resistance. Samples clustered by genome were not excluded, provided these had been assayed independently.  Ethics statement  Approval for the CRyPTIC study was obtained from the Taiwan Centers for Disease Control IRB 106209; University of KwaZulu Natal Biomedical Research Ethics Committee (BE022/13); University of Liverpool Central University Research Ethics Committees (2286); InstitutionalResearchEthicsCommitteeofTheFoundation for Medical Research, Mumbai (FMR/IEC/TB/01a/2015 and FMR/IEC/TB/01b/2015); Institutional Review Board of PD Hinduja Hospital and Medical Research Centre, Mumbai (915-15-CR); scientiﬁc committee of the Adolfo Lutz Institute (CTC-IAL 47-J/2017); the Ethics Committee (81452517.1.0000.0059) and Ethics Committee review of Universidad Peruana Cayetano Heredia (Lima, Peru); and London School of Hygiene & Tropical Medicine (London, UK). |
|  | See Online for appendix 2 |  | Methods  Data sources  In this systematic analysis, we collected existing worldwide MTBC WGS data and associated phenotypic DST data from academic groups and consortia, reference laboratories, public health organisations, and published literature (appendix 2 table S1). Data were | |  | Phenotypic data  We collected both categorical (resistant or susceptible) and quantitative (minimum inhibitory concentrations [MICs]) phenotypic data. Diﬀerent DST methods and resistance-deﬁning critical concentrations were accepted. To ensure comparability and optimise quality, we categorised phenotypes as follows: methods and |

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