Impact of Whole-Genome Sequencing of *Mycobacterium Tuberculosis* On Treatment Outcomes for MDR-TB/ XDR-TB: A Systematic Review Protocol

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Protocol

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Abstract

Background

The emergence of drug-resistant tuberculosis (DR-TB) is a persistent threat to public health. The detection of DR-TB requires culture-based drug susceptibility testing (DST) or rapid molecular assays for targeted genes. The recent advances in Whole-genome sequencing (WGS) technology have offered a new capacity to identify resistance-conferring mutations in Mycobacterium tuberculosis (MTB). This study reviews and quantifies the emerging evidence on the association between genomic markers of drug resistance in MTB identified by WGS and treatment outcomes for DR-TB.

Methods

A literature search will be conducted in NCBI PubMed, Scopus, Cochrane Library, Web of Science, and CINAHL (Ebsco) to retrieve all the relevant original reports from 2000 onwards. Clinical trials and observational studies describing different applications of WGS to genotypic resistance testing for TB and detection of MDR-TB/ XDR-TB as well as treatment outcomes of the patients will be included. Two primary reviewers will separately screen and select papers for data extraction, risk of bias, and assess the quality. Any disagreement between the reviewers' will be clarified by a third reviewer. The I^2 statistics will be used to assess the heterogeneity of the included studies and if the data are sufficiently homogenous, a meta-analysis will be performed. The Egger's test and visual representation of the funnel plot will be used to monitor for publication bias. Narrative data synthesis will be conducted for all the included studies if performing meta-analysis is not possible.

Discussion

This systematic review will examine the evidence on the feasibility and added value of WGS in improving treatment outcomes in DR-TB patients. The rapid detection of drug-resistance conferring mutations and selection of appropriate drug regimens is likely to improve the cure rates while minimizing adverse events and treatment costs. Hence, the outcome of this systematic review will inform policy-making and will guide clinical laboratory practice to improve drug resistance diagnostic capacity and treatment outcomes.

Systematic review registration:

PROSPERO CRD42020197099.

Introduction

Despite the global efforts, drug-resistant tuberculosis (DR-TB) remains a major obstacle in tuberculosis (TB) eradication programs. Globally in 2019, the World Health Organization (WHO) reported around 465,000 new cases and approximately 182 000 deaths due to multi-drug resistant TB (MDR-TB)/ rifampicin-resistant TB (RR-TB) [1]. As per WHO, 17.7% of prior treated cases and 3.3% of new patients had RR/MDR-TB, and successful treatment outcomes were accounted for only 57% of cases with RR/MDR-TB [1]. *Mycobacterium tuberculosis* (MTB) isolates resistant to first-line drugs isoniazid and rifampicin are defined as MDR-TB, and extensively drug-resistant TB (XDR-TB) are referred to as MDR isolates, along with resistance to a fluoroquinolone and another Group A drug (e.g., linezolid or bedaquiline) [2–4]. The detection of DR-TB using culture-based drug susceptibility testing (DST) can be challenging. It remains time-consuming, expensive, and requires a high-capacity laboratory, as well as methodological standardization of testing for second-line antimycobacterial agents [5, 6]. In 2019, only 71% of RR/MDR-TB cases were tested for second-line drug fluoroquinolones, which could be due to inaccessibility of test facilities or culture of slow-growing MTB for phenotypic DST [1].

Currently available genotypic assays can rapidly provide common drug resistance which is advantageous over time-consuming phenotypic DST; however, these diagnostic methods can only detect specific genetic mutations already known to confer drug resistance [7, 8]. Whole-genome sequencing (WGS) has become a reliable tool to overcome the limitations of probe-based diagnosis methods and provide a better insight into emerging clinically relevant mutations in the MTB genome [9, 10]. WGS is relatively rapid and has the capacity to identify a broad range of genetic polymorphisms, which can guide us to new treatment strategies and improve case management for DR-TB [8, 11]. The WGS -based applications are expanding to TB diagnostics and recent studies demonstrated the applicability of WGS in the clinical management of tuberculosis [10, 12, 13]. Also, previous systematic reviews of Mycobacteriology have emphasized the application of WGS from a different perspective. The use of WGS for TB transmission and outbreak investigations was reviewed by Hatherell HA [14] and Van Der Werf MJ et.al. [15], whereas, Papaventsis D et.al [8] and Nieto Ramirez LM et.al. [16] addressed the diagnostic accuracy of WGS for detecting the mutations conferring drug resistance. This systematic review will bridge the knowledge gaps on the efficacy of MTB whole-genome sequencing on patients' treatment outcomes.

The standardized treatment approach for individual MDR-TB cases does not always lead to successful treatment outcomes. As several patients with a history of TB treatment develop MDR/XDR TB, baseline drug resistance has been recognized as a major factor that impacts the treatment efficacy [17]. Therefore, in this present review, we aimed to assess the applicability of WGS to detect genomic markers of drug resistance in *M. tuberculosis* and explore their association with treatment outcomes.

Objectives:

To evaluate the efficacy of whole-genome sequencing as a predictor of treatment outcomes for multidrug-resistant/extensively drug-resistance tuberculosis compared to phenotypic drug-susceptibility testing.

Methods

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols (PRISMA-P) statements were used to develop this systematic review protocol [18] (Additional file 1). This present protocol was registered with the International Prospective Register of Systematic Reviews (PROSPERO) database (registration ID: CRD42020197099).

Eligibility criteria

We will include non-Randomized clinical trials (NRCT), Randomized clinical trials (RCT), and observational studies, such as case-control, cohort, case series describing different applications of WGS to genotypic resistance testing for TB and detection of MDR-TB/ XDR-TB as well as treatment outcomes of the patients. The exclusion and inclusion criteria of this study are defined using PICOS (Population, Intervention, Comparator, Outcome, Study design) framework [19] (Table 1).

Table 1
Inclusion and exclusion criteria.

PICOS criteria	Inclusion	Exclusion
Population	Studies conducted on MDR-TB/ XDR-TB groups	Studies reported any single drug resistance TB group e.g. RR-TB group
Intervention	Whole-genome sequencing	Targeted genome sequencing
Comparator	Phenotypic DST	
Outcome	Studies reported MDR-TB/ XDR-TB treatment outcomes	
Study design	Original researches: clinical- trials and observational studies like case-control, cohort, case series	Conference abstracts, reviews, letters to editors, editorials

Search strategy

Five electronic databases will be used to conduct a literature search i.e. Scopus, Cochrane Library, Web of Science, NCBI PubMed (MEDLINE), and CINAHL (Ebsco). The structured search strategy will be employed based on the keywords related to “drug resistance tuberculosis”, ‘tuberculosis’, “treatment outcome” and “whole genome sequencing”. Also, the site-specific biomedical terms like Medical Subject Headings (MeSH), Emtree will be included and the Boolean operators will be used to combine all of the search terms [20] (Additional file 2). The search data will be reviewed and validated by a specialist health science librarian. The results will be limited to the English language and original research articles from the year 2000 onwards will be included. Letters to the editor, review articles and comments will not be included in this study. There will not be any geographical filter as the review intends to include global data.

Selection of studies

The search results retrieved from the electronic databases will be imported in Zotero and duplicate studies will be removed. For the title-abstract screening phase, the retrieved studies will be exported to a Microsoft Excel spreadsheet (Microsoft, 2019). Two reviewers will independently assess the abstracts using eligibility criteria. Studies identified as relevant by at least one of the reviewers will be included for full-text assessment. The full text will be retrieved and assessed by two reviewers independently. Any disagreement between reviewers' interpretation will be initially discussed between them, and, if not resolved, will be clarified by a third reviewer. The final decision of including/ excluding a study will be confirmed by all reviewers and appropriately documented in the PRISMA flow chart [21].

Data extraction: Pertinent data will be extracted separately by the primary reviewers from each included article. A standardized data extraction spreadsheet will be developed by the reviewers after a pilot assessment. The key details to be extracted will include the following: Basic details (i.e. names of authors, year of publication, journal, the country where patients were enrolled in the study, duration of the study, study design, study setting, method of WGS employed in the study), study participants (number of patients included, age, gender, risk groups when available), main findings (MTB isolates subjected to phenotypic DST and WGS, MDR-TB/XDR-TB numbers, mutations detected by WGS, technical details about diagnostic tools, MTB genetic lineages), treatment outcomes (treatment completed, cured, died, defaulted, TB relapse).

Risk of bias and quality assessment

The quality assessment of included studies will be performed using the Cochrane Risk of Bias 2 (RoB 2) tool for RCT [22], Cochrane Risk of Bias in Non-randomised Studies of Interventions (ROBINS-I) tool for NRCT [23] Newcastle-Ottawa Scale (NOS) for quasi-experimental and observational studies [24] or based on the study designs. Two reviewers individually will determine the risk of bias and based on the study design, studies will be classified as having a ‘low,' ‘high,' or ‘unclear' risk of bias. A third reviewer will address any discrepancies between the primary reviewers.

Data synthesis and analysis

The study characteristics and outcome data will be collated into tables and summarised narratively. The I^2 statistics will be used to assess the heterogeneity of the included studies and if the data are sufficiently homogenous, a meta-analysis will be performed. Random or fixed-effect meta-analysis will be carried out to estimate the individual study effect and produce a pooled estimate summarizing data on the predictors of all distinct treatment outcomes [25, 26]. Standardized mean differences will be used to assess continuous outcomes and odds ratio (OR) and Risk ratio (RR) will be used to estimate the effect sizes of categorical outcomes, with a 95% confidence interval (CI) [27]. The degree of heterogeneity among the studies will be represented visually using a forest plot [28] and the value of the I^2 statistics and the Egger's test and visual representation of the funnel plot will be used to assess publication bias [29, 30]. Narrative data synthesis will be conducted for all the included studies if performing meta-

analysis is not possible. We will adhere to the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach to assess the data quality presented in the systematic review [31, 32]. Reporting of the systematic review and meta-analysis-PRISMA 2020 guidelines will be used to report this systematic review [21].

Subgroup analysis

A subgroup analysis will be conducted, in cases of a significant heterogenicity observed in pooled estimates, considering the following grouping characteristics [27, 33]

i. Type of drug-resistant strains of MTB: MDR-TB, XDR-TB.

ii. Participants demographic and risk factors: age, HIV status.

iii. Study characteristics: geographical area and study design.

iv. Types of sequencing platforms and data analysis tools.
v. Participants' treatment outcomes: treatment completed, cured, died, defaulted, TB relapse.

Discussion

Despite recent advances in the application of WGS to the prediction of drug resistance in MTB, the association between detection of resistance or susceptibility from genome sequence data and the treatment outcomes in the case of DR-TB/ MDR-TB/ XDR-TB remains untested. This systematic review will interrogate the existing data on the feasibility and added value of WGS in improving treatment outcomes in TB patients. The rapid detection of drug-resistance conferring mutations and selection of appropriate drug regimens is likely to improve the cure rates while minimizing adverse events and treatment costs for TB patients. Hence, the outcome of this systematic review will be beneficial for policy-making and will guide clinical laboratory practice to improve drug resistance diagnostic capacity and treatment outcomes. While the genomic sequencing methods and bioinformatics analyses employed in studies included in this systematic review will not be specifically scrutinised, we acknowledge the importance of wet and dry laboratory techniques and analytic pipelines in the implementation of WGS-guided selection of therapy for DR-TB [34]. We will pay particular attention to the interpretation of drug-resistance mutations, their impact on phenotypic DST results, and the ranking of their significance and will recommend a minimum data required for a standardised assessment of genetic mutation-clinical outcome associations in order to avoid biases. In addition, this review will shed light on important challenges in the translation of genomic research into TB control and personalised therapeutic interventions and identify relevant research gaps for further studies.

List Of Abbreviations

DR-TB: Drug-resistant tuberculosis; TB: Tuberculosis; WHO: World Health Organization; RR-TB: Rifampicin resistant tuberculosis; MDR-TB: Multi-drug resistant tuberculosis; MTB: *Mycobacterium tuberculosis*; XDR-TB: Extensively drug-resistant tuberculosis; DST: Drug susceptibility testing; WGS: Whole-genome sequencing; Preferred Reporting Items for Systematic Reviews and Meta-Analyses: PRISMA; RCT: Randomized clinical trials; NRCT: Non-Randomized clinical trials.

Declarations

Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable.

Availability of data and materials: Not applicable

Competing interests: The authors declare that they have no competing interests.

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Authors' contributions:

The study was conceptualized by VS, KC, DH, CL and supervised by KC, VS, VSD, and BTV. The search strategy was designed by DH, VSD, and CL, and the literature review was performed by DH and CL. The manuscript has been written by DH and reviewed and improved by VS, KC, VSD, CL, and BTV. All authors critically revised the current protocol and approved the final version of this manuscript.

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