In Focus

A Collaborative Approach for “ReSeq-ing” *Mycobacterium tuberculosis* Drug Resistance: Convergence for Drug and Diagnostic Developers

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Since 2002 there has been a gradual, 1.3% per year, decrease in the incidence of tuberculosis (TB) worldwide. Though this is encouraging, this level of reduction will not meet the goal of the World Health Organization (WHO) to eliminate TB by 2050. In addition, the problem is only being compounded by the growing incidence of drug-resistant tuberculosis (Dye et al., 2013). Keys to the effective control of TB and the spread of drug resistant strains include the quick and accurate procurement of drug susceptibility information from patient samples that are infected with *Mycobacterium tuberculosis* complex (MTBC), linkage to appropriate treatment regimens, and follow-up to ensure cure (Wells et al., 2013). Phenotypic culture-based solutions, although the current “gold standard”, are cumbersome, time-consuming, and consequently have a high percentage of loss to follow-up. Furthermore, culture-based solutions expose laboratory workers to potential risk of infection and certain drugs have assay reproducibility and accuracy concerns. Moreover, there are a multitude of phenotypic tests using different methods and growth media which result in discordant phenotypic results depending on the drug being tested. Detection of resistance concurring mutations by molecular methods is a promising alternative, but there is a paucity of information that correlates the infecting MTBC strain’s genotype to phenotype and ultimately to patient outcomes (Noor et al., 2015).

To take better advantage of the present and emerging knowledge on genetic patterns encoding for drug resistance, there is a need to establish a sustainable data sharing platform and associated bioinformatic analysis tools. The platform will need to be able to house genotypic data, its related phenotypic data and associated metadata from individual isolates and be easily maintained, accessed and queried. The platform should contain the current wealth of knowledge that is located in disparate databases located in multiple research centers from around the world. But it will also be critical to validate the consistency and quality of the data added to the platform, and organize and maintain contemporaneous validated datasets. This will be especially important for data related to off-label use of repurposed drugs currently used to treat TB (Sotgiu and Migliori, 2015).

To prevent drug selection pressure from nurturing the development of resistance in treated populations, it will be imperative to align the roll out of new drug (Evangelopoulos and McHugh, 2015) and drug regimens (Fig. 1) with novel diagnostic tools to monitor drug resistance. Furthermore, drug developers need to revise their informed consent in these trials so that MTBC isolated from treated patients can be used in research to further dissect clinically relevant drug resistance mechanisms. The proper management of drug resistance is more cost effective than developing new anti-infective drugs and will secure the efficacy of these newly developed antimicrobials for the future.

The currently available genotypic and correlated phenotypic data concerning the resistance of MTBC have not been vetted or validated in a standardized manner, nor have the multiple data sets been merged. Moreover, many of the data sets are not curated (in terms of quality control) and may not be properly maintained. Clinical information (e.g., treatment outcome) is present only sporadically. Therefore, the first challenge lies in the development of a well-structured data platform that integrates high-quality sequencing data using a unified standardized pipeline from different research centers together with the relevant complementary information including associated drug susceptibility testing phenotypes, minimal inhibitor concentrations, resistance associated mutations, single nucleotide polymorphisms (SNPs), and additional clinical outcome data. While a limited set of genetic resistance markers for tuberculosis drugs has been identified, knowledge gaps still exist which impede the broader use of genomic information for the prediction of drug resistance (Salamon et al., 2015).

With the introduction of new drug combinations and regimens, and patients with potentially more complex resistance profiles, it is imperative to understand SNPs and genotypic information, and their correlation with phenotypic resistance, in order to allow for the implementation of correct, personalized therapies in a rapid, individual
been developed in a phased manner with the phenotypic and associated meta-data for MTBC. The initiative has a data sharing platform which will be used to aggregate genotypic, and associated meta-data for MTBC. The initiative has been developed in a phased manner with the first set of objectives centered on the design and development of the platform and the expansion of an initial characterization of SNPs that have been observed in MTBC. Raw sequencing data obtained from contributors will be curated, annotated, globally aggregated datasets which can be utilized for database interrogation, pertinent statistical data correlations, and meaningful data outcomes and applications. As a further incentive, their contributions will result in individual and institutional researcher acknowledgement by way of credit within relevant publications.

Accelerated development of the proposed advanced bioinformatic tools for use with the ReSeqTB platform is possible due to the complementary skills of the partners in the initiative bringing together subject matter experts in data warehousing, scientific experts, and end users who will test and improve the tools in an iterative manner. Plans are also in development to allow researchers access to raw data to accelerate the development of new products and to improve case management in the clinical setting. The ReSeqTB platform can be utilized to minimize diagnostic costs, advance the research agenda and reduce testing times which could ultimately benefit patients, through advances in basic research (sequencing of resistance genes) to clinical research (identification of new drug targets), new diagnostic products (faster diagnosis of resistant bacteria and viruses), improved medical treatment (avoidance of antibiotic resistance due to inadequate drugs and dosages) and reduced health expenditures on a national level (avoidance of expensive 2nd and 3rd line drug treatments) (Table 1).

Initial results are already indicating new, but also more complex answers to the question of the genetic foundations of antibiotic resistance, as demonstrated by the discovery of additional putative genes and the indication of intergenic non-coding regions associated with drug resistance (Walker et al., 2015). The ReSeqTB platform will make it possible to better understand the underlying biology governing complex resistance profiles, to foster development of more powerful and targeted treatments, to foster development of more powerful and targeted
Table 1  
Stakeholder needs and benefit analysis.

| Stakeholder | Interests | Needs | Benefits |
|-------------|-----------|-------|----------|
| Patient     | Proper, accurate and effective treatment of their MTBC infection | Rapid development and deployment of assays for the accurate and sensitive detection of MTBC resistance | Faster and more accurate diagnosis of drug resistant MTBC |
|             |           | New and more effective therapeutic regimens for resistant MTBC and for decreasing the potential for developing resistance | More appropriate and effective therapeutic regimens for drug resistant MTBC |
|             |           | More effective therapeutic regimens for non-resistant MTBC which decreases the potential for resistance | More effective therapeutic regimens for non-resistant MTBC |
| Individual academic data contributor | Basic research in drug resistance mechanisms | Access to high quality, curated, annotated, globally aggregated data sets for research purposes | Virtual collaborative environment |
|             | Basic research in drug development | Ability to utilize the data sets for new investigations | Co-authorship on output which utilizes their data |
|             | Access and utilization of high quality, curated, annotated, globally aggregated data sets for research purposes | Access to results of raw data processed through the unified pipeline | |
| Pharma co. data contributor | Access and utilization of high quality, curated, annotated, globally aggregated data sets for research purposes | Ability to analyze sequence data from clinical trials | |
|             | Access to high quality, curated, annotated, globally aggregated data sets for research and development purposes | Access to data analysis tools (long term deliverable) | |
| DST developer end user | Validated list of resistance associated mutations | Access to high quality, curated, annotated, globally aggregated data sets for research and development purposes | Consensus driven interpretation of MTBC sequence data |
|             | Interpretation/meaning of resistance associated mutations | Access to the results of raw data processed through the unified pipeline | Ability to data mine an MTBC sequence database of high quality curated annotated globally aggregated data sets |
| Research end user | Access and utilization of high quality, curated, annotated, globally aggregated data sets for research purposes | Ability to compare analysis data against a validated set of resistance associated mutations for MTBC | |
|             | Access to high quality, curated, annotated, globally aggregated data sets for research and development purposes | Access to data analysis tools for new investigations (long term deliverable) | |
| Treatment advocacy group | Patient treatment with the ‘best’ regimen possible based upon the patient’s sequence data | Validated resistance associated mutation list that is updated as often as possible and which develops the list for new drugs and drug regimens prior to their general implementation | Proactive database of validated resistance associated mutations (updated prior to release of new drugs and drug regimens) |
| Funding organizations | Impact on funded projects | Guidance information and materials to allow for proper assessment of proposals | Validated, consensus driven database and analysis pipeline for funded proposals in MTBC sequencing projects |
| High burden and low burden country’s ministries of health | Tools for increasing the effectiveness of treatment in such a manner that improves overall treatment outcomes and decreases the economic impact of treatment | Decreasing the rate of infection for MTBC and drug resistant MTBC | Potential for beneficial economic impact on the diagnosis and treatment of drug resistant MTBC |
|             |           | Tools for increasing the effectiveness of treatment | Validated list of resistance associated mutations with associated reference and supporting data |
|             |           | Active MTBC surveillance | Increased impact with sequence based surveillance studies |
drugs, to provide timely and personalized therapies and to contribute to current surveillance efforts. Ultimately, ReSeqTB will help to fight the further spread of drug resistant strains within a community by facilitating the potential for guided and personalized therapeutic regimens which cannot be achieved with presently available approaches.

Disclosure

The authors declared no conflicts of interest.

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