COMMENTARY

Finding Achilles heel of drug resistance in *Mycobacterium tuberculosis*

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Tuberculosis (TB), a pulmonary disease caused by *Mycobacterium tuberculosis* is known to be the second largest cause of death and poses a serious threat to public health throughout the world. Although it can affect immunocompetent people, individuals with weakened immune system (e.g. HIV infection) are at an increased risk.

India bears the highest burden of TB globally; about 25% of all cases are reported here. The extent of TB in high-burden, low-resource regions is difficult to measure directly; but knowing the number of cases is necessary to plan, staff and finance the control programmes. In 2015, a total of 1.6 million Indians were notified with relapse and newly diagnosed TB and at least 480,000 Indian citizens died from the disease. TB can affect people of any age, caste or class; but occurs mainly in poor people. Slum-dwellers, load-bearing workers, rickshaw-pullers, construction workers, malnourished individuals and those with compromised immune system are more at risk of contracting TB. Children comprise 40% of the population, but are currently possibly under-diagnosed with TB in India. The World Health Organization (WHO) estimated that nearly 1 million people with TB in India are not notified. Simple health sector interventions have been inadequate to stem this staggering toll. Diseases such as TB are not just a social deterrent, but are also a strain on the economic growth of the country. Thus, there is a need for complete assessment of the impact of TB, not simply to inform policy-makers of the burden of disease, but also to provide the basis for target interventions in TB control.

A consistent and comparative description of the burden of diseases and injuries, and the risk factors that cause them is an important input to decision-making and planning processes in the health sector. Information that is available on mortality and health in populations in all regions of the world is fragmentary and sometimes inconsistent. Thus, a framework for integrating, validating, analysing and disseminating such information is useful to assess the comparative importance of diseases and injuries in causing premature death, loss of health and disability in different populations.

Over a timespan of 15 years between 2000 and 2015, despite strengthened monitoring, reporting and treatment efforts, TB continues to be an economic burden. In 2015, it accounted for a burden of US$ 20 billion[1]. The economic burden has been calculated on the basis of the WHO's standard metric of disability adjusted life years (DALYs) method. The exercise shows that there is a need to look beyond the gaps in public health system measures, and focus on discovery and development of new drugs for TB. The upstream science needs attention to be capable of delivering newer therapies for treating TB. There is a need to involve and leverage the role of information and communication technology (ICT). The actions needed to address the issue of TB burden in India include:

(1) Monitoring the compliance of TB-infected patients to the treatment regime. An extensive epidemiological study for Multi Drug Resistant (MDR), Extreme Drug Resistant (XDR) and relapse cases is warranted.

(2) Evolve preventive mechanism of disease infection in populations identified as high-risk, e.g. construction workers, mine workers, load-bearing workers, rickshaw-pullers, slum-dwellers, malnourished and immuno-compromised people, etc.

(3) Intensify action for developing new drugs, rapid diagnostics and effective vaccines.

The treatment for TB infection begins with the first-line drugs which are administered for a continued period of 6–9 months. However, treatment with these drugs often fails to cure TB for various reasons, including non-compliance with the drug treatment and prolonged use of multiple antibiotics. The emergence of MDR-TB and XDR-TB is of great concern, as these can lead to persistently progressive disease with a high morbidity and mortality rate. The treatment of MDR-TB and XDR-TB is excessively long compared to drug-susceptible TB, and may require the use of second-line drugs that are expensive, difficult to procure and more toxic than first-line drugs. Drug resistance and tolerance by the bacterium are the Achilles heel of *Mycobacterium tuberculosis* (*Mtb*) infection. Therefore, there is a need to understand the complex biological responses of this bacterium in order to enhance the process of drug discovery.

The unmet medical need for TB prompted us to propose a novel integrated methodology that employs systems-level analysis, genome-scale variation analysis of the clinical isolates, followed by structure-wise selective chemical tailoring of molecules for the predicted non-toxic metabolic targets in *Mtb*.

Previously, systems biology spindle map (SBSM) approach was being used to identify potential non-toxic drug targets required for the growth and survival of *Mtb*, which is the basis of the present study. The 890 *Mtb* metabolic genes, including 116 *in silico* essential genes (75% of which are experimentally validated) and 211 metabolic persister genes (MPGs), were analysed for their invariance. Among the 116 essential genes obtained from *in silico* gene knockout, 104 were found to have no homology to human genome sequences. Of these, a total of 25 genes showed no variation across the 1084 MDR *Mtb* Russian strains and only 8 showed one non-synonymous variation[2].

Genes with restricted variations are more likely to be essential for survival of the organism and to be ideal targets for drugs. It was observed that each of the invariant genes had a unique target metabolite. This makes each of these genes unique in its action and also makes it likely that there is no genetic redundancy. Therefore, if a drug is designed against these essential genes, it will remain highly specific in the inhibition of metabolic pathway by effectively acting on them. The specific functionality of these genes ensures that the functioning of the drug will not bring about any other stochastic damage and will be highly exclusive in its action.

Finally, the 33 genes that were categorized as invariant can be taken up as potential drug targets for MDR-TB. Since drug resistance is a major concern, we evaluated mutations in the targets of the

first-line drugs. Our analysis showed that isoniazid, pyrazinamide and ethambutol targets exhibited a high degree of mutations in the clinical isolates genome, specifically in the binding pocket of the targets, thereby resulting in drug resistance.

atpE, the target for bedaquiline which is a new drug for *Mtb* showed no variation in the entire 1623 *Mtb* strains, thus validating our approach for selection of potential targets which are evolutionarily conserved. According to recent reports, lansoprazole, a well-known proton pump inhibitor drug for acid reflux (novel class of cytochrome bc1 inhibitor), exhibited good *in vitro* and *in vivo* activity against *Mtb*. According to our analysis, the target for lansoprazole showed very few mutations in the entire *Mtb* strains, further strengthening the hypothesis. Recently, we have proposed that metformin, a popular drug for type-II diabetes can also inhibit bacterial NDH- I which is encoded by *nuoA* (*Rv3145* in *Mtb*), thereby opening up possibilities of adjunct therapy for TB[3]. We observed that the target for metformin showed no mutation in 1084 Russian MDR strains.

Therefore, it is clear that the understanding of mutation sites in the protein structure of existing drug targets from genome sequence analysis of clinical isolates is crucial for identifying the origin of drug resistance and availability of any other binding site for drug design. In this context, we have analysed the intracellular levels of all the proteins coded by the 33 invariant genes based on the complete proteome of *Mtb* as reported recently. Also, the druggability assessment of these 33 targets suggested that 22 of them are highly druggable. Out of these 33 targets, 15 (Rv2763c, Rv3247c, Rv1094, Rv3607c, Rv3048c, Rv2965c, Rv2361c, Rv0865, Rv0321, Rv0098, Rv0390, Rv3588c, Rv2244, Rv2465c and Rv2607)[2,4] have available crystal structures in the Protein Data Bank (PDB). Upon screening, it was observed that 7 of the 15 have reported GSK inhibitors. This approach was extended towards identifying small molecular candidates against *Mtb*, which led to 20 novel leads including four FDA-approved drugs (droxidropa, tetroxoprim, domperidone and nemonapride) for which the validatory assays were available[2,4]. We therefore expect that identification of these repurposed drugs using systems-level analysis can function as crucial tools in targeting *Mtb*. Therefore, it is proposed that this comprehensive integrated methodology, with both experimental and *in silico* approaches, has the potential to not only tackle the MDR form of *Mtb*, but also the most important persister population of the bacterium. Based on the existing knowledge, we propose that the most critical targets in a pathogenic organism (e.g. *Mtb*) should be evolutionarily conserved and functionally critical[2].

A recent study, using the aforementioned systems biology approach, has identified and experimentally validated pranlukast (PRK) as a highly effective drug against *in vitro* and *in vivo* survival of *Mtb*, and being an FDA-approved drug, it shows the potential for development of advanced combinatorial therapy against TB[5].

This novel approach of *in silico* drug designing, based on integrated experimental data, will reduce the cost of drug discovery by lowering the chance of failure at the clinical trial level. In recent years, several drugs have been discovered against TB including bedaquiline[6] (TMC-207), delamanid[7] (OPC-67683), gatifloxacin[8], moxifluxacin[9], PA-824 (ref. 10) and rifapentine[11]. However MDR-TB treatment still remains a challenge. PA-824 standalone is not effective, while bedaquiline has serious side effects. As previously stated, metformin has been proposed as a potential combination therapy along with existing frontline antibiotics for TB[4]. Even at the present rate of drug discovery, there exists the need for potential drugs and drug targets to cope with MDR-TB and XDR-TB. The demand for potential drugs and targets against drug resistance TB can be hopefully met, given the recent systems biology methods used to identify evolutionarily conserved, novel, non-toxic targets, coupled with proper understanding of the various complex interactions between the pathogen and its human host.

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