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Tackling tuberculosis: insights from an international TB Summit in London

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†SB was the chairperson on the March 24th (World TB Day), "TB Summit 2015."

Keywords: antibiotic resistance, diagnosis, drug susceptibility testing, latency, *Mycobacterium*, new drug discovery, repurposing drugs, tuberculosis, treatment, vaccine

Abbreviations: BCG, Bacillus Calmette-Guérin; BPA, Bangladesh Pediatric Association; CNS, Central Nervous System; DST, Drug Susceptibility Testing; EMB, Ethambutol; FBG, Fasting Blood Glucose; IFNγ, Interferon gamma; IgG/A, Immunoglobulin G/A; IGRA, Interferon Gamma Release Assays; INH, Isonicotinoyl hydrazide (Isoniazid); LTBI, Latent TB Infection; MDR, Multidrug Resistant; MRC, Medical Research Council; NAAT, Nucleic Acid Amplification Test; NAT, Arylamine *N*-acetyltransferase; NSAIDs, Non-Steroidal Anti-Inflammatory Drugs; NTP, National Tuberculosis Control Program; PCR, Polymerase Chain Reaction; PZA, Pyrazinamide; RBG, Random Blood Glucose; RIF, Rifampicin; TA, Toxin-Antitoxin; TB, Tuberculosis; TBM, TB Meningitis; TNF α – Tumor Necrosis Factor alpha; TST, Tuberculin Skin Test; VBNC, Viable But Not Culturable; WGS, Whole Genome Sequencing; XDR, Extremely (/Extensively) Drug Resistant; WHO, World Health Organization

Tuberculosis (TB) poses a grave predicament to the world as it is not merely a scientific challenge but a socio-economic burden as well. A prime cause of mortality in human due to an infectious disease; the malady and its cause, *Mycobacterium tuberculosis* have remained an enigma with many questions that remain unanswered. The ability of the pathogen to survive and switch between varied physiological states necessitates a protracted therapeutic regimen that exerts an excessive strain on low-resource countries. To complicate things further, there has been a significant rise of antimicrobial resistance. Existing control measures, including treatment regimens have remained fairly uniform globally for at least half a century and require reinvention. Overcoming the societal and scientific challenges requires an increase in dialog to identify key regions that need attention and effective partners with whom successful collaborations can be fostered. In this report, we explore the discussions held at the International TB Summit 2015 hosted by EuroSciCon, which served as an excellent platform for researchers to share their recent findings. Ground-breaking results require outreach to affect policy design, governance and control of the disease. Hence, we feel it is important that meetings such as these reach a wider, global audience.

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Introduction

Continuing its strong association and commitment toward bringing attention to the infectious disease regarded as the scourge of the poor and a health risk globally, EuroSciCon (http://euroscicon.com/) held its third international TB Summit in East London, UK.[1,2] In this report, we strive to provide an overview of the main topics and the key ideas covered by the oral and poster presentations as well as the thought-provoking discussion sessions that were a part of each day of the conference. The three day conference brought together world-leading scientists, clinicians, representatives from the industrial sector, students and post-doctoral fellows for intensive sessions that aimed to bring out emerging issues on tuberculosis (TB) that require immediate global attention.

The importance of these annual events cannot be overlooked as the death toll due to TB still averages at 1.5 million lives every year.[3] Although the causal organism of the disease, *Mycobacterium tuberculosis*, was reported more than a century ago, scientific progress has not matched up to its adaptability and versatility in order to put this menace to an end. A three-pronged approach of prevention, accurate diagnosis and effective treatment is essential to overcome this disease.[4] However, there are many bottlenecks that impede development in all 3 areas. In the absence of validated, reliable biomarkers to judge the protective efficacy and safety of new vaccine candidates we are left with Bacillus Calmette-Guérin (BCG), the only vaccine available for the last hundred years, even though it falls short of protecting populations against active pulmonary TB.[5]

With more than a third of the world's population latently infected with TB, any chemo-prophylactic measures including vaccines, that could assist the host's immune system to remove these dormant pathogen cells, is appreciated as crucial. Latent TB infections (LTBI) are a potential health threat as they may

revert to active infections due to immune-compromisation of the primary host. However, molecular details of the host-pathogen interplay that are instrumental in bringing about the physiological switch are still largely unknown.[6] For hundred years, the only diagnostic technique to detect LTBI was the tuberculin skin test (TST) until recently when interferon gamma (IFNγ) release assays (IGRA) were licensed for use. On the other hand, chest X-rays, smear microscopy and sputum culture are the classical diagnostic techniques to detect active TB. However, these methods make great demands on labor, time and resources. This is more so when culture methods are used for drug susceptibility testing (DST). With the advent of nucleic acid amplification tests (NAAT), such as the GeneXpert®, diagnosis of drug resistance has become faster, however deployment of the PCR-based system in resource-poor settings is still a distant goal.[7,8]

Misdiagnosis of the disease most often results in inappropriate therapy which further exacerbates the issue of drug resistance in the pathogen. Inadvertent mistakes in treatment can lead to selective pressures which are beneficial for strains that are already drug resistant or ones which have a greater propensity to acquire them. Thus, what was once considered a consequence of patient non-compliance or a healthcare management-related issue is now forcing us to rethink the '*magic pill*', standardised strategy of overtly depending upon antibiotics. As deaths due to multi-drug and extensively drug-resistant (MDR and XDR) TB keep increasing the arsenal of novel therapeutic targets and new classes of antibacterial compounds needs to keep up the pace accordingly.

Rise of the 'super strains'

Mycobacterium tuberculosis, the causal organism of TB is presumed to have originated in the Fertile Crescent and the areas neighboring the African continent and migrated from there.[9] Two of the 7 currently existing lineages of the pathogen still remain in Africa, while the modern lineages have infiltrated Africa by thoroughfare of infected individuals.[10]

The rise and spread of antibiotic resistance have very rightly captured the attention of the masses. Drug-resistance in TB has been rising and spreading throughout the world so much so that there is at least one report of XDR-TB in 100 countries worldwide at this present moment.[3] Though many reasons have been attributed to this rise of resistance, most of these have been related to practices such as patient habits, incorrect diagnoses and breakdown of nationalised healthcare systems.[11] However, advances in genotyping and strain identification have indicated the predominance of some lineages over others in relation to their ease of transmission to susceptible hosts.[12] These fast-spreading modern lineages have not only surpassed the ancient ones in numbers but also appear to acquire resistance very successfully, thereby deserving immediate attention as treatment options remain limited.[13] Dr R. Anthony, Royal Tropical Institute (The Netherlands), a regular participant of the conference, divulged details of his group's investigation into the drug-resistance related mutations in the Beijing-type strain of the pathogen. They generated several mutants *in vitro* that were resistant to the isoniazid (INH), commonly used to treat TB. *M. tuberculosis* develops drug resistance exclusively through chromosomal mutations, in particular single-nucleotide polymorphisms.[14,15] However, he doubted that the mutants that are raised against a single drug in the laboratory are, for the most part, representative of the resistant organism infecting the host. Many of these lab-based mutants cannot survive in a human patient as they either lose their virulence or pathogenicity.[16] Thus, there must be a low number of mutations through which it is possible to retain pathogenicity and yet gain resistance. To detect these, the line probe assays were used. Other than being elegant and simple, these are highly sensitive in picking up specific alterations in selected genes. It was observed that clinically relevant Beijing-type mutants, when exposed to selective pressure by exposure to rifampicin (RIF) a first-line anti-TB drug, were more successful in developing resistance to it compared to their non-Beijing counterparts. A study in the Republic of Georgia, wherein numerous strains were genotyped also showed a high association of multidrug resistance with Beijing strains.[17] The interaction between Beijing strains and RIF using specialized membrane-culture methods teamed with customised microscopy revealed that these strains may be intrinsically more insensitive toward the drug than the other strains.[18] This along with the previous findings that these strains acquire mutations readily has severe implications on whether the standardised TB regimen as accepted and prescribed worldwide is indeed falling short, and instead of curbing, is actually fuelling the growth of resistance.[19] He advocated the need to look at the interactions in the genomes, especially into why some strains are more suited to existing with the mutations and find it easy to accumulate and survive the defects related to these abilities.

Moving toward the host's end of the spectrum, this disease manifests itself in several forms - latent, pulmonary and disseminated, to name a few. Strain association with the disease form has been noted as in the case of Beijing strains being incriminated for a majority of the disseminated TB cases. An investigation to characterize the predominant strains of *M. tuberculosis* infecting the population in the Amhara region of Ethiopia was undertaken by Dr S.A. Yimer *et al.*, Oslo University Hospital (Norway).[20] A cross-sectional study of 240 sputum samples revealed the Central Asian strain to be the most predominant. However, the most striking finding of the study was the discovery of lineage 7 in this region. Whole genome sequencing (WGS) of lineage 7 places it between the modern and ancient lineages. The lineage has been stable over a long period of time and has started to diversify only recently for reasons unknown. Patient information reveals that this strain is associated with those who show delay in seeking treatment.[20] This strain grows slower *in vitro* as compared to the other strains and the lack of pathogenicity resulting in milder symptoms which may explain the delay of patients in seeking treatment. However, it is this shortcoming that gives the strain an advantage over others as untreated patients keep transmitting it further as is evidenced by clustering of incidences by Tessema *et al.*[21]

Yet another burning issue is that of the lack of a simple, inexpensive, gold standard test for the detection of pyrazinamide (PZA) resistance in *M. tuberculosis*. PZA was introduced to anti-TB therapy in the 1980's. It targets the dormant or 'persister' subset of the infecting microorganisms and was thereby crucial in

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shortening the therapy course from 9 months to the current 6-months regimen.[22,23] Though its mechanism of action and development of resistance against it is fairly well-understood, identifying drug resistant cases is still cumbersome.[24] Dr D.A. Maslov, Russian Academy of Sciences (Russia) and team, undertook a study to understand the prevalence of PZA-resistance in the MDR population of the European region of Russia. Three different techniques, namely the BACTEC™ MGIT™ 960 PZA Kit, Wayne PZase activity assay and sequencing the genes controlling PZA resistance (*pncA, panD, rpsA*), were used to identify the resistant mutants.[25,26] Although all the techniques missed a few cases, detection by BACTEC™ MGIT™ remained the method of choice as it had the best combination of sensitivity and selectivity compared to the others. Among the 519 MDR isolates collected in this study, over 70% were found to be PZA-resistant (unpublished data). Interestingly, the authors found one PZA-resistant isolate lacking mutations in all 3 known PZA-resistance associated genes.

Thus, there is a need for tight connection between genomic and microbiological level investigations that can serve to feed information to each other. A concerted approach is of paramount importance to understand this multi-faceted organism better and to reveal the chinks in its armour that can be exploited for diagnosis as well as therapy.

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TB and its murky associates

The high rates of TB co-infection in HIV positive patients has been established by studies conducted in Africa which bears a high burden for both the diseases.[27] On the other hand, diabetes mellitus predisposes the patient to develop active TB and severely affects the treatment outcome such that individuals suffering from the former condition are twice as likely to die from the latter disease.[28,29] Diabetes, a condition usually identified with affluent countries now also has a greater stronghold in low and middle-income countries where HIV and TB are already rampant.[30] Dr A. Wafi, Aligarh Muslim University (India) spoke about the significance of Th1 and Th17 cells as markers in TB patients with diabetes mellitus. Both are subsets of T-cells and are strongly involved in the host's cell-mediated immunity and produce inflammatory effectors, or cytokines as a response to external agents. Elevated levels of these T-cells and IFN-γ were found in patients suffering from both diabetes and TB when compared to those with only TB. It was suggested that diabetes is associated with alteration of the immune response to TB leading to a biased treatment outcome. Thereby prophylactic treatment against TB to all diabetic patients with LTBI was recommended by the speaker.

WHO (http://www.who.int/en/) and The Union (http://www.theunion.org/) together, have published policy documents regarding the key guidelines for further research to strengthen the understanding of the association between diabetes and TB. However, none of the meta- and systematic reviews available in published literature includes African populations. Though there is no reason to suspect a difference of the disease associations in different ethnicities, the high prevalence of HIV in these populations does present a further complication. Dr S. L. Bailey, London School of Hygiene and Tropical Medicine (UK), has been looking into the modifying effect of HIV on the association between hyperglycaemia and active TB in Lusaka, Zambia. A case-control study sampled 3000 TB and 7000 non-TB (sputum culture negatives) cases obtained from the regional TB clinics of 3 communities. Diabetes among these individuals was diagnosed via RBG (random blood glucose) concentration and the results showed that a low percentage of the TB patients examined were hyperglycaemic and a similar proportion was found among the controls. This low prevalence of hyperglycaemia goes against the trends seen elsewhere; there seems to be no association between TB and hyperglycaemia in the HIV negative patient group. This is sharply reversed in the HIV positive group where a strong association is seen between the 2. Only at elevated levels of hyperglycaemia (>11.1 μmol/L) was the difference really noticeable. However, newly diagnosed TB-patients, often weakened by the disease condition commonly exhibit a stress-response, which can manifest itself as hyperglycaemia.[31,32] As a consequence it was necessary to discriminate between the proportion of the patients that were hyperglycaemic due to diabetes mellitus and those with stress-induced hyperglycaemia. A subset of the previously studied TB cases was selected and RBG, FBG (fasting blood glucose) and HbA1c concentration at the time of TB diagnosis was measured. As a reference standard test, FBG concentration was measured post initiation of treatment. Comparison of the different diagnostic results showed that roughly half of the RBG and FBG-detected hyperglycaemia cases were diabetes related. These results show that most patients with HIV had stress-induced hyperglycaemia. In light of this information, the initially striking correlation of the HIV positive diabetics with active TB infections proved to be insignificant. Overall, the study revealed no evidence of association between hyperglycaemia and active TB in sub-Saharan Africa. These findings are in stark contrast with the TB-diabetes relation found in other parts of the world.[33-35]

Not only do other diseases put patients at risk of contracting TB but those with active pulmonary TB have increased chances of developing mycosis as well. Co-infections with *Cryptococcus spp*., *Coccidiomyces spp* and *Histoplasma spp*. have been recorded since the late nineteenth century. Whether the infections occur concurrently or sequentially does not affect treatment the outcomes.[36] However, diagnosis and treatment of these infections together is complex as discussed by Professor R.P. Mendes, UNESP (Brazil).[37] As the symptoms of most of these fungal infections imitate that of pulmonary TB, these may lie undetected for a while. Once identified however, initiation of the classical anti-TB and anti-fungal therapy usually results in a worsening of symptoms as these drugs interfere with each other. Drug replacements such as using moxifloxacin instead of rifampicin or rifabutin and itraconazole for posaconazole were suggested. However, treatment of TB in these cases remains of utmost importance as control of its transmission is a common, global priority. Emphasis was laid on expanding our understanding of the drug-interactions and increasing the arsenal of drugs currently available today.

Tubercular infection of the central nervous system (CNS), often known as TB meningitis (TBM) is a serious and life-threatening condition. It accounts for up to 5–10% of all the TB cases

worldwide but is responsible for more than 40% of the deaths due to TB.[38] TBM is characterized by varied clinical manifestations such as, those without tuberculoma or infarct; those with either tuberculoma or infarct; and those with both. Patients with tuberculoma are difficult to treat because the disease persists even with anti-TB therapy and invasive treatment can lead to severe complications. Data regarding prognostic value of tuberculomas has been limited with conflicting evidence.[38,39] It is believed that patients with tuberculoma have the worst prognosis but Prof. M. Wasay, Aga Khan University (Pakistan) pointed toward several other factors that were better indicators of treatment outcomes in these cases. Though factors such as hydrocephalus, advanced age and active TB infection appeared to indicate poor treatment outcomes; he observed that infarctions, either chronic or acute, serve as very good determinants for prognosis. Follow-up MRI scans to monitor the evolution and progression of the disease phase may help in identifying new infarctions and the response to medical intervention. Prof. Wasay recommended stroke prevention in CNS TB as the most important area requiring consideration as a reduction in this can substantially lower the number of deaths owing to CNS TB. The role of aspirin and high dose steroids in CNS TB has been debated.[40,41] The predisposing and protective factors for tuberculoma formation in CNS TB are little understood. In addition, the duration of anti-TB treatment varies case-by-case and little is known about stroke prevention in these cases. The speaker raised the questions in areas where there is little knowledge regarding strain, virulence, resistance patterns, host factors associated in increased risk for CNS TB. He called for more randomized control trial studies to be done and increase in collaboration, funds and capacity for this area to improve understanding and ultimately improve management of CNS TB.

Zoonotic nature of the *Mycobacterium* species

The pathogenic species of *Mycobacterium* are notorious for causing zoonoses, infectious diseases of animals that can be spread to humans.[42] *M. bovis* is an infectious, slow-growing pathogen responsible for causing TB in cattle and is also a part of the *M. tuberculosis* complex, causing the disease in man.[43] Due to the development of pasteurisation techniques, the incidences of TB in humans due to *M. bovis* has decreased substantially over the years. However, this disease continues to plague cattle, with UK having the highest bovine TB incidence in all of Europe. There has been a significant increase of bovine TB cases from 1076 in 1996 to 4720 infected animals in 2014.[44] An estimated half of these herds are infected by badgers (*Meles meles*) that serve as the natural reservoir of this pathogen. The first badger harbouring *M. bovis* was reported in the early 1970s.[45] Since then, the Badger Protection Act (consolidated in 1992) has served to protect and preserve this species of animal.[46,47] Currently, UK has the highest concentration of badgers in Europe and up to 30% of the badgers in a region can be expected to harbour the TB bacilli. The route of transmission of the bacteria to cattle has not been established yet.

Vaccination of cattle is illegal in the EU, as after administration of BCG, there are no means to discern between a vaccinated and an actively infected animal due to the cross-reactions routinely faced while using the TST as a diagnostic tool.[48] Therefore control measures are limited and usually involve annual surveillance, removal of infected animals and control of cattle movements (physical barriers).[48] Badger vaccination is suggested by Dr S. Lesellier, Animal and Plant Health Agency (UK), as a means to reduce the prevalence and severity of bovine TB in a badger population and thereby reduce the rate of transmission in cattle. BCG vaccine has been proven safe in humans; it is inexpensive and readily available. However, acquiring a license (obtained in 2010) for its use in badgers required 10 years of exhaustive studies to demonstrate the safety and efficacy data for using BCG in captive and wild badgers.[48] Demonstration of safety and efficacy of the vaccine was done by 2 studies that used 17 intra-muscularly and/or subcutaneously vaccinated badgers among which 8 were given the dose allowed for humans and the rest received a higher dose.[49] Six unvaccinated badgers were used as the controls. Seventeen weeks after vaccination, the badgers were infected with *M. bovis* directly *in situ* by endobronchial instillation in the right middle lung lobe using a flexible fibroscope. Samples taken for immunology and culture were removed at regular intervals and 12 weeks after infection the animals were sacrificed followed by detailed post mortem examination. A statistically significant reduction in the disease burden was observed when higher dose was used for vaccinating the badgers. Excretion of *M. bovis* from the trachea was also found to be lower in number and appeared later in the timespan of the infection. Badger-specific IGRA assays revealed higher levels of IFN-γ in the animals that received a higher dose. However, post-challenge the levels of IFN-γ normalized for all probably making a case that it may not be a reliable marker to estimate protection.

The observational studies to collect the safety and preliminary efficacy data from the wild badger population spanned over 3 years: animals were trapped, vaccinated or kept as non-vaccinated controls, microchipped for identification and monitored over time. No animal was sacrificed, therefore post mortem data are not available, and instead 3 live tests were conducted. A high prevalence of TB was found in the badger populations pre-study, but a highly significant reduction of up to 3-quarters was observed in incidence at social group levels. Further analysis of data identified an indirect, protective effect in unvaccinated cubs when more than a third of their social group had been vaccinated. This is probably due to the *'herd effect'*, but the same wasn't recorded in adult badgers. As seen with other species, BCG, though effective, is not a 100% eliminator of the risk of infections. However, a 75% protection rate justified the issue of a license for the use of BCG in badgers. The next progression of this study would be to develop an oral vaccine for badgers. This would remove the need to trap and vaccinate individual badgers while reaching a larger number of animals and could be potentially cheaper. However, this will require an oral vaccine that produces consistent protection in badgers, equivalent to that seen with the injectable vaccine. In addition, palatable bait, the best means to deploy the vaccine for maximum uptake and the implications of this vaccine on human health and the environment need to be established. Though there are oral vaccine-candidates

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available, obtaining a license and transferring the technology to a manufacturer for scaling up production will still require a good part of a decade.

It is speculated that TB may have infected the mastodon, however the first established case was reported in 1875 at the London Zoo.[50] By the late 20th century it was recognized worldwide though no studies have been done to estimate their global prevalence. The rise of TB in captive elephants in the USA was discussed by Dr J. Landolfi University of Illinois (USA). Presently, there are 55 culture-confirmed cases of TB in elephants in the United States of America. A majority of these cases are due to *M. tuberculosis* and sparingly due to *M. bovis*. The infection is manifested as a chronic, subclinical infection with severe dissemination. Unfortunately, most cases only get documented post mortem. As a result, about 65% of the infected animals are now deceased. In addition to the concern regarding the health of the animals, there are strong indicators that it may be transmissible from the handlers and vice versa. The high prevalence of TB among humans in Southeast Asia indicates that in this case, it may be that humans serve as the reservoir infecting the Asian elephants, which appear to be more susceptible to it. Differences in the immune functions of this population of elephants may serve to explain the presence of a special susceptibility toward the pathogen. However, issues regarding the lack of reliable diagnostics, efficacious treatment regimens and understanding of the mechanism of the underlying disease appear to be universal across host species.

For an understanding of the physiology of the disease progression, Dr Landolfi's group looked at formalin-fixed, paraffin-embedded sections of lung from captive north American and Asian elephants.[51] Latent lesions were found to be well organized, discrete granulomas, with many activated macrophages and CD3+ T-cells. Histologically, in the active TB cases there were widespread, poorly granulomatous lesions. Inflammation was found to be centered on regions of necrotic debris with a low number of macrophages and lymphocytes mostly of the B-cell type.

Reverse transcriptase PCR to detect the mRNA levels of cytokines from unstimulated peripheral whole blood samples from 106 captive elephants was studied.[52,53] No statistically significant differences were observed between the TB positive and negative elephant samples. However, incorrect diagnosis while selection of the elephants, lack of information on the disease stage and the use of unstimulated blood could have had an impact on the results seen. As an improvement, mycobacterial antigens such as PPD, and CFP10 were used with ConcA as a control to stimulate peripheral blood derived monocytes. These studies showed elevated mRNA levels of TNF α, IFN γ as well as IL-17 in the TB positive elephants with PPD-B acting as the best stimulant.[54] Detection of TB in elephants relies on trunk wash procedures as both TST and X-ray analyses are not feasible. Though serodiagnosis is being pursued now, the low and availability of these tests remain an issue. Dr Landolfi hopes for the whole blood assay to have similar or better sensitivity and specificity than the current gold standard technique, the trunk wash, which is a labor intensive method. She warned that the advancements in detection of the disease would have to be paralleled with assessment techniques for response to treatment as well as development of a more suited treatment regimen for elephants.

Understanding the human response to TB: insights for diagnosis and monitoring patients

Rapid assessment of the response to TB treatment in humans at present is a considerable road-block and accurate methods to do so can have a definitive impact on treatment outcomes. Cytokines play a major role in protection against mycobacterial infections and regulate the immune response at a cellular level. TNF α, IL-12 and IFN γ are the common cytokines that have been investigated *in vitro* but there are very few *in vivo* studies carried to assess their role as early treatment biomarkers for MDR-TB. N Fatima, Aligarh Muslim University (India) took up a study to determine the correlation between cytokine levels in the pulmonary and extra-pulmonary TB patients. A total of 65 blood samples were collected from a cross-section of TB infected patients and controls. The results showed an increased TNF-α level was seen in new TB cases and in MDR patients meanwhile no significant rise of IL-4 in new TB patients.[55] The analysis of the various cytokines revealed that these cellular responses may indeed be different in new TB, MDR patients and patients with extrapulmonary TB. Hence, these could serve as biomarkers for the response to TB treatment and is therefore important for both clinical practice (in the detection of MDR TB cases) and clinical trials of new anti-TB drugs.

Exploiting the often ignored humoral immune response to mycobacterial antigens was suggested by Emeritus Professor PK Das, University of Amsterdam (The Netherlands). He pressed that these responses can be identified by electrophoretic techniques and can help in differentiating between mycobacterial infections and also detecting the stage of the disease.[56] The differences in the type (IgG, IgA) of antibody and their pattern as seen on electrophoreses, can conclusively predict infection by mycobacteria.[57]

One of the greatest impeding forces to vaccine developments against TB is the lack of known antigens that could selectively prime the host's immune system toward pathogenic mycobacteria. Alternatively, identifying a response to a specific antigen in an infected individual could also serve as a robust diagnostic tool.

In an effort to identify potential antigens, Dr AJ Minnaard, University of Groningen (The Netherlands) extracted all the lipids of the outer layer of mycobacteria and identified those present only in the pathogenic species.[58,59] These glycolipids get picked up by our immune systems, especially by the CD1 system producing immunogenic responses and thereby providing protection to the host.

These compounds are difficult to extract due to the slow growing rate of the pathogens, but can be prepared organically. All compounds contain a *polar head* such as a trehalose, mannose or a complex tri-saccharide. They also comprise of a *non-polar tail* which is very waxy in nature. New synthesis tools incorporating new catalysts had to be developed for their synthesis.[60] In an iterative process the stereo methyl groups were added with a quality check at every step followed by extensive characterization to

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confirm the product.[61,62] This effort required 40 years since the isolation of the compound from the mycobacterial cells and has currently received support from the Bill and Melinda Gates Foundation.

One natural product of *M. tuberculosis*, the 1-tuberculosinyladenosine (1-TbAd) lipid is composed of a diterpene unit linked to adenosine. A screen for 1-TbAd mutants, complementation studies, and gene transfer identified Rv3378c as essential for 1-TbAd biosynthesis.[63] This gene is associated with virulence and its deletions fail to prevent lysosomal maturation and acidification in macrophages.[64]

A strong, fundamental understanding of the host-pathogen interactions taking place during infection is essential to inform both diagnostic and preventative therapeutics research. The antigen profiles of the pathogen at its various physiological stages need further elucidation as does the cytokine, serological and cellular responses of the host.

Surgical interventions for alleviation of TB

In the 1880s, thanks for Carlo Forlanini, lung collapse therapy or artificial pneumothorax, became the treatment of choice for pulmonary TB as the world still awaited the discovery of antibiotics.[65] As antibiotic resistance now grips the globe, attention is being re-focussed on the role of surgery in the management of active TB infections.

Extra-pulmonary TB in the thoracic cage can include infections of the lymph nodes, the pleura, chest wall, and so on. Detection of these cases is difficult and often tissue diagnosis is required. Though the role of surgery in TB remains mostly diagnostic, Prof K.M. Al-Kattan, King Faisal Specialist Hospital & Research Center (Saudi Arabia) suggested using it for treatment as well as palliative treatment. Several procedures such as simple drainage to more complicated ones such as wedge excision lobectomy, pneumonectomy, mediastinal lymphadenopathy and thoracoplasty were discussed. As surgery is an extreme measure and it is mandatory that the patient has been on anti-TB therapy for at least 3 weeks prior to the operation. This route is undertaken only when there are added complications such as non-responsive MDR TB housed in fibrotic structures that preclude treatment or involvement of fungal infections. However, in spite of all standard precautions being taken, morbidity due to blood loss and post-op complications remain high. In some cases though, surgery is inevitable as infections of the chest wall result in eroded sternums that require complete restructuring. Enlarged lymph nodes in children can compress the mediastinum and in these cases surgery can only partially resolve the condition.

Arthritic TB of the spine was first described by the British surgeon, Percivall Potts and is now known as the Pott's disease. It is a secondary localization of pulmonary TB, which in its acute phase forms abscesses capable of causing damage to the vertebrae and discs. It can lead to severe vertebral deformities (scoliosis and kyphosis) which may result in disabling compression of the spinal cord and neurological complications.

It is an extrapulmonary form of the disease that results in excessive back pain leading to tissue and vertebral damage. The best remedy for these cases, as advocated by Dr S. Rigotti, Ospedale Sacro Cuore (Italy), is a strut grafting procedure as it allows for healing and spinal fusion required for maintenance of spinal stability (kyphotic angle). Early detection is the key for success of this procedure. Once detected through MRI, percutaneous abscess drainage procedure is carried out and the fluid obtained is sent for DST. Following the operation, long bed rest of up to 12 months is necessary followed by the use of a brace for another 18 to 30 months. Anti-TB therapy comprising of an initial 2 months of RIF, INH, EMB, PZA, followed by 10 months of only INH and RIF is usually followed. Once the infection has been treated, an operation to correct the effects of drainage on the spine has to be performed. The stabilization operation involves screws inserted into the vertebrae. An extended follow-up period of 2 years ensures total recovery of saggital balance and frontal stability of the patient. The procedures are minimally invasive with little risk to adults, however the situation becomes complicated in children below 12 years of age as their bones are not ossified and infection is usually detected much later in its progression.

Treatment of TB: successes and failures

Though an established and effective treatment against the susceptible forms of TB exists, the decline of the deaths due to TB seems to have plateaued in recent years.[66] This could be because of the emergence of the drug resistant variants of the pathogen, which claim the lives of around half of the patients they infect depending upon the diagnostic and treatment facilities available to the patient.[67–69] Though MDR TB remains a minor portion of the total TB worldwide, the rate of notified cases has remained unchanged over the past 5 years at 0.3 per 100,000. As diagnosing resistance is especially difficult, this may not reflect the real numbers. While discussing their successful results of MDR/XDR TB treatment, Dr T. Van der Werf, University of Groningen (The Netherlands), mentioned the need to incorporate the treatment outcome results into scientific analyses. He brought to attention that MDR is not a homogenous disease and shows a gradient of severity which, in effect, progressively restricts the available treatment options. Further complications arise from the fact that the dose of drugs achieved in patients varies from one to the other. Insufficient drug exposure or inadvertent monotherapy result in emergence of resistant organisms, an event made more likely when using a relatively weak second line drug.

Using a treatment structure that was individualised for patients but still following WHO guidelines, Dr Van der Werf reported a favorable outcome in 85.6% of patients treated for MDR-TB between 2000 and 2009 using 18 month treatment plans. These included using higher doses of class 5 drugs than conventional practice, including linezolid and Clofazimine in the regimen, the latter of which was described by Dr Van der Werf as a promising addition. Therefore, based on their promising outcome results he suggested the use of drugs at doses above the recommendations and accompanied by close observation of the patient.[70,71] Unknown drug interactions are one of the reasons a '*one size fits all*' treatment regimen does not work. Highlighting the lack of randomized, controlled trials to guide antimicrobial treatment for MDR TB, Dr van der Werf mentioned that the

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single best predictor for efficacy of any antimicrobial drug is evaluation of the drug exposure relative to susceptibility of the organism.

Anti-TNF therapies for rheumatism have been very effective and have led to the development of several artificial monoclonal antibodies such as infliximab and rituximab that act as TNF agonists. However, TNF is pivotal in the immune response to *M. tuberculosis* and helps contain the infection.[72] Therefore, the use of these agonists may increase the risk of patients developing TB or other complications from infections by non-tuberculous mycobacteria. Keane *et al.* showed that there were 70 reported cases of TB, usually extra-pulmonary, from the 147,000 patients administered with infliximab worldwide between 1998–2001.[73] Almost all of the TB cases were reported in countries with low incidence of TB making this association a highly alarming one. Etarnecept, another TNF antagonist was found to fare marginally better than infliximab and adalimumab, but the TB incidences still remain high.[74,75] Professor O.M. Kon, Imperial College Healthcare NHS Trust (UK), highlighted the probability of most of these cases being LTBI that are activated as the host immune surveillance drops and recommend testing for it before initiating treatment for rheumatism. Therefore diagnosing LTBI in these patients is a high priority. Patients are usually risk-assessed, taking into account the risk of prophylaxis versus developing TB and then put forward for diagnosis. However, risk stratification misses 2-thirds of the cases thereby lowering the sensitivity manifold. For diagnosis, chest X-rays, TST and IGRA are the methods of choice. The TST can be used for diagnosis but patients with rheumatism usually have an attenuated response to the test.[76] IGRAs in this case are highly specific for the detection of LTBI unless steroids are administered to the patient. In the absence of guidelines for practitioners, Professor Kon suggested a thorough follow-up and surveillance of patients by taking into account their contact and travel histories as treatment with biologics has been noted to be associated with reactivation of TB many years post cessation of treatment.

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Treatment of TB: a new way forward

As the pathogen develops new mechanisms to get past the drugs meant to kill it, development of antibiotics with novel mechanisms of action become imperative. With a view to exploit the mycobacterium cell's requirement for iron, Dr M.J. Miller, University of Notre Dame (USA) discussed the potential of species-specific siderophores or mycobactins as potential antibiotics.

Mycobacterial cells have an obligatory requirement for iron (III) which is assimilated from the environment with the help of siderophores secreted by the cells. All organisms have slightly different siderophores with mycobacteria having 3 types.[77] Interestingly, these siderophores, or mycobactins from one organism can antagonise the growth of another and thus show potential for use as antibiotics.[78] Additionally, attaching a deleterious group to the mycobactin of a species converts it into a *Trojan horse* that serves to kill the organism effectively as seen when artemisin is attached to mycobactin T.[79,80] These antibiotics are extremely specific, however synthesising them is not practical. Thus, high-throughput screening of small fragments of the mycobactins and their derivatives were undertaken. Only the oxazolines formed from the fragments showed activity against the pathogen. Surprisingly, these compounds had no role in iron transport. They were also found to be metabolically unstable.[81] The amides of these compounds were more stable but lost their activity against *M. tuberculosis*. Investigations of the structure activity relationships led to the synthesis of pyridine compounds that are metabolically stable with highly improved activity. Over 500 analogs of the compounds have been synthesized and have been found to have very good biological activity against drug resistant strains of the pathogen as seen in mouse models.[82] The active compounds are believed to target cytochrome C oxidase and shut down respiration of the mycobacterium. The low MICs in the nanomolar concentrations and the reasonable space to tune the compounds to improve their pharmacodynamic/kinetic properties do indeed raise hope for a new drug against TB in the near future.

Looking to plants for inspiration, Dr M. Baltas, The Center National de la Recherche Scientifique (France) and his group, investigated the potential of cinnamic acid derivatives. One of the derivatives incorporates isoniazid and another has a styryl triazolophtalazine frame. The latter showed very good activity even against INH-resistant *M. tuberculosis*. Following these successes, a 2-step library of around 200 compounds were made containing aromatic rings with different linkers and hydroxyl groups. Triazoles, representative of the triclosan systems, and pyrrole derivatives were made and found to have similar activity against the pathogen.[83,84] Treatment of mycobacteria with these compounds, specifically, their lead compound TB09 reduced the cell wall mycolates thereby showing evidence that these compounds act on trehalose dimycolate and interfere with the cell wall assembly.

Natural products are also an extensive source of novel chemical scaffolds from which novel anti-tubercular agents with pleiotropic modes of action can be discovered as was discussed at the poster presentation sessions.[85–90] To considerably shorten the time of development of a molecule from bench-to-bedside another feasible strategy is to repurpose existing drugs used clinically for other conditions or comorbidities. The potential of repurposing non-steroidal anti-inflammatory drugs (NSAIDs) as anti-tubercular therapy was discussed and also gathered serious consideration.[91-95]

Moving on to fragment-based approaches to develop inhibitors of mycobacterial growth, Dr E. Polycarpou, Kingston University (UK), spoke about inhibitors designed against enzymes required for cholesterol metabolism. Cholesterol is required during various stages of the bacterial life cycle-phagocytosis, bacterial inhibition of lysosome maturation and bacterial replication within macrophages.[96] Transcriptomic and gene deletion mutant studies reveal *hsaD* gene in the cholesterol catabolism operon in *M. tuberculosis* to be essential for survival within macrophages and therefore a key target.[97] HsaD is a stable enzyme that cleaves the C-C bond through serine/threonine protease-like catalytic triad.[98] Mechanism-based inhibitors of the enzyme showed poor selectivity and/or aqueous solubility and therefore a fragment-based approach was adopted. The rationale behind this approach is to identify low affinity scaffolds that can then be combined to

increase selectivity and effectiveness of the inhibitors designed. A library of 1260 fragments was screened using differential scanning fluorimetry to evaluate their binding affinity to HsaD. Seven initial hits were then verified using a high-throughput colorimetric assay developed in their lab. X-ray crystallography enabled in resolving the structures of 2 fragments and understanding the binding sites of the fragments on the enzyme. Structure-activity relationship further helped refine the fragment structures to inhibit mycobacterial growth specifically while being non-toxic to mammalian cells and *E. coli*.

Arylamine *N*-acetyl transferase (NAT) was found to be present in humans serving to detoxify drugs.[99] It was also found to be present in mycobacteria where it appears to play a significant role in mycolic acid synthesis and the proper assembly of the cell wall.[100] A rapid, colorimetric screening method to detect the activity of the enzyme enabled screening of a large number of fragments.[101,102] Two promising fragments were identified, one being a prodrug and the other binding directly to the enzyme and faring better at eukaryotic cell toxicity than the former. The latter compound was found to be more effective than INH and a few derivatives have been designed. The NAT inhibitors are currently under license to Eli Lilly from Summit PLC.

Toxin-antitoxin (TA) modules comprise of 2 genes where the upstream antitoxin abrogates the effect of the cognate toxin which targets an essential cellular function. The post-genomic era has revealed that these modules are diverse and ubiquitous. These TA are found in pathogenic mycobacteria, except *M. leprae* but not found in non-pathogenic ones. 88 putative TA systems have been identified in *M. tuberculosis* H37Rv out of which 40 have been found to be functional. ParDE2 is a putative TA module that was investigated by M. Gupta, Jawaharlal Nehru University (India). RNA transcript analysis of the *M. tuberculosis* demonstrated that the genes were transcribed and hence not pseudogenes. In cells overexpressing only the toxin ParE2, growth was inhibited but the cells remained alive thereby becoming viable but non-culturable. Microscopic images of these cells revealed elongated phenotypes with an extended periplasmic space and corrugated rough membranes enclosing several vacuolar structures.

ParE2 was expected to target the DNA gyrase mechanism. DNA gyrase is made up of 2 subunits, A and B that uncoil the DNA molecule by cleaving one strand passing it around the other and ligating it back. Purified mycobacterial DNA gyrase subunits A and B were obtained and investigations with the holoenzyme revealed that the toxin most likely interferes with the ligation step. The C-terminus of ParE2 was found to interact with GyrB and a 20 amino acid deletion abrogated its toxicity. When transformed *M. smegmatis* cells containing the TA module were used to infect THP-1 cells, those expressing the wild-type toxin showed lowered bacterial loads within the macrophages. On the other hand, the ones with truncated versions of the toxin showed cell viability at par with the non-transformed controls. This points to the hypothesis that, under stress conditions the bacteria produce proteases that act upon the antitoxin thereby allowing the toxin to have its effect on the cell which shifts to a VBNC phenotype inside the macrophage. Additionally, ParE2 expressing *E. coli* cells demonstrated increased tolerance toward antibiotics, surviving well over the established minimum inhibitory concentrations.

The growth of antibiotic resistance and the subsequent shortfall in the number of effective drugs available for treatment provided the much need incentive for expanding research into antimicrobial drug discovery and design. It is encouraging that scientists have identified and are investigating several alternative routes to identify these molecules starting from the microbes themselves to plants and synthetic molecules.

Raising consciousness toward TB: in public and specialists alike

The TB burden in children is estimated to be in the range on 4–21% of the total burden depending on the country.[103] Bangladesh, a high burden country, reports only around 3% of its total disease burden owing to childhood TB.[104] This discrepancy suggests that Bangladesh fails to detect TB in approximately 15,000 children annually. A survey of the regions showed that the sub-district hospitals had low rates of diagnosis probably owing to the low clinical capacity and lack of skilled healthcare workers in the regions. Therefore in 2012 a guideline was laid out for the training and modular capacity-building of doctors and health care workers. It contained training modules, facilitator's guides, flip charts and training videos. The objectives and achievements of this approach in Bangladesh were shared by Dr S. Ahmed, Shaheed Suhrawardy Medical College (Bangladesh).

The main challenges in developing the modules were taking into account the varied age and skill ranges that the materials had to cater to. A comprehensive and generic training module was drafted based on the local case data available from the country's records. With the support of USAID under the guidance of National Tuberculosis Control Program (NTP), Bangladesh Pediatric Association (BPA) TB care II started the training from November, 2013. Seventeen districts and 122 sub-districts servicing a population of around 150 million were covered. The module was designed to have various components such as, personal reading, peer reading, group tasks, case demonstration and case solving. All the participants of the training went through a pre-training test and post-training test comprising of 20 questions. The results of the 2 tests showed a significant increase in knowledge after the training sessions irrespective of the initial skill, experience and age of the participants. The training was completed on 2nd July 2014 and records since then show that childhood TB diagnoses in Dhaka have increased. However, in some areas the detection has gone down or is still far from the expected numbers showing that training can resolve this issue only in part and that a reliable diagnostic tool for those below 4 years of age is necessary.

Developments in any field of research require a steady inflow of investments and grants. To achieve the decrease in the rates of mortality and incidences that have been set as goals by the Stop TB partnership (http://www.stoptb.org/) and the WHO, greater investments are required. This can be attracted only be raising and sustaining the profile of TB by ensuring heavy political involvement. The cost of antimicrobial resistance is estimated to be around US$ 100 trillion by 2050 as highlighted by Dame

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Sally Davies, the Chief Medical Officer of the UK. A similar cost analysis of TB including the cost to the agriculture industry could be the key to grabbing public attention. A systemic analysis of TB research in the UK in the period between 1997 and 2013 was carried out by Dr. M. Head, Infectious Disease Research Network (IDRN, UK; http://www.idrn.org/) and his collaborators. Funds received by UK institutions for infectious diseases research was mapped for an initial period between 1997–2010.[105] This included human infections only, unless zoonosis was demonstrated. The data was sourced directly from the donor's records and the awards were normalized by converting international currency to GBP sterling and adjusted for inflation. Studies were categorised based on the disease area, clinical specialty, specific infection, R&D value chain (clinical trials phases, etc). Around 7400 grants were awarded over 1997–2010 and 1228 from 2011–2013 with a total investment of approximately ≤3.7 billion. Medical Research Council (MRC), UK and the Wellcome Trust were revealed as the top donors while HIV and malaria received the most funding. This is disparate to the disease burden, where TB research received only 6% of the total amount awarded.

In the initial years of the analysis, pre-clinical science received higher funding but there appears to be a slight shift toward funding the clinical phase trials of drug development in the recent years. More awards for translational research, product development, diagnostics and vaccine therapy was observed as well.

As a leading investor in global infectious disease research, UK has been investing relatively well in TB but an increase in funding is required. A look into the costs, burdens and the funding biases of other world economies in infectious and non-communicable diseases could inform policy makers and funding bodies and assist in the identification of research gaps and priorities.

Conclusion

Battling this scourge of the past that has firmly found a place in the current times requires co-ordinated action from workers in the laboratory to those out in the clinics. This meeting nurtured intense and focussed scientific dialog that promises to seed long-lasting collaborations, or at the very least, raise awareness and educate. Impassioned pleas from all the speakers resonated the similar theme that prevention, diagnostics and treatment need to be strengthened. As fundamental research gains focus, it is important to note that antibiotic resistance in itself is on its way to far greater notoriety than TB and needs immediate attention from policy makers, pharmaceutical companies and scientists alike. It is encouraging that early-stage researchers are acknowledging the need for increased interdisciplinary approaches and communication. Noble efforts such as the IDRN (UK) aimed at increasing the capacity of research need to be applauded and supported.

Advances in the field of vaccine development are apparent in that 11 candidates that were either BCG replacements or boosters were in clinical trials in 2010.[106] In the absence of reliable biomarkers to predict the protection offered by vaccines, large-scale human efficacy trials is the only option to evaluate a vaccine. These studies need to be further refined as there are many factors such as age, ethnicity, gender bias of the cohort that could affect the outcome of the trials.

Under or mis-diagnosis of TB has been instrumental in the spread of MDR- and XDR-TB. The Stop TB Partnership has thus called for immediate action for the production of new diagnostic techniques for rapid identification of drug susceptible and resistant TB as well as LTBI.[107] Dorman suggests moving from culture-based techniques as any such tool will only produce incremental gains on the currently existing methods of diagnosis.[108] With the advent of the IGRAs, PCR- and line probe-based assays, the search for diagnostics appear to be on track.

Finally, after half a century of no new drugs to treat TB, the drug development pipeline now contains a number of novel and repurposed drug candidates.[109] This improvement of the situation is however, far from the one desired considering the high attrition rates of candidates in drug trials as well as the well-equipped opponent we are faced with.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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