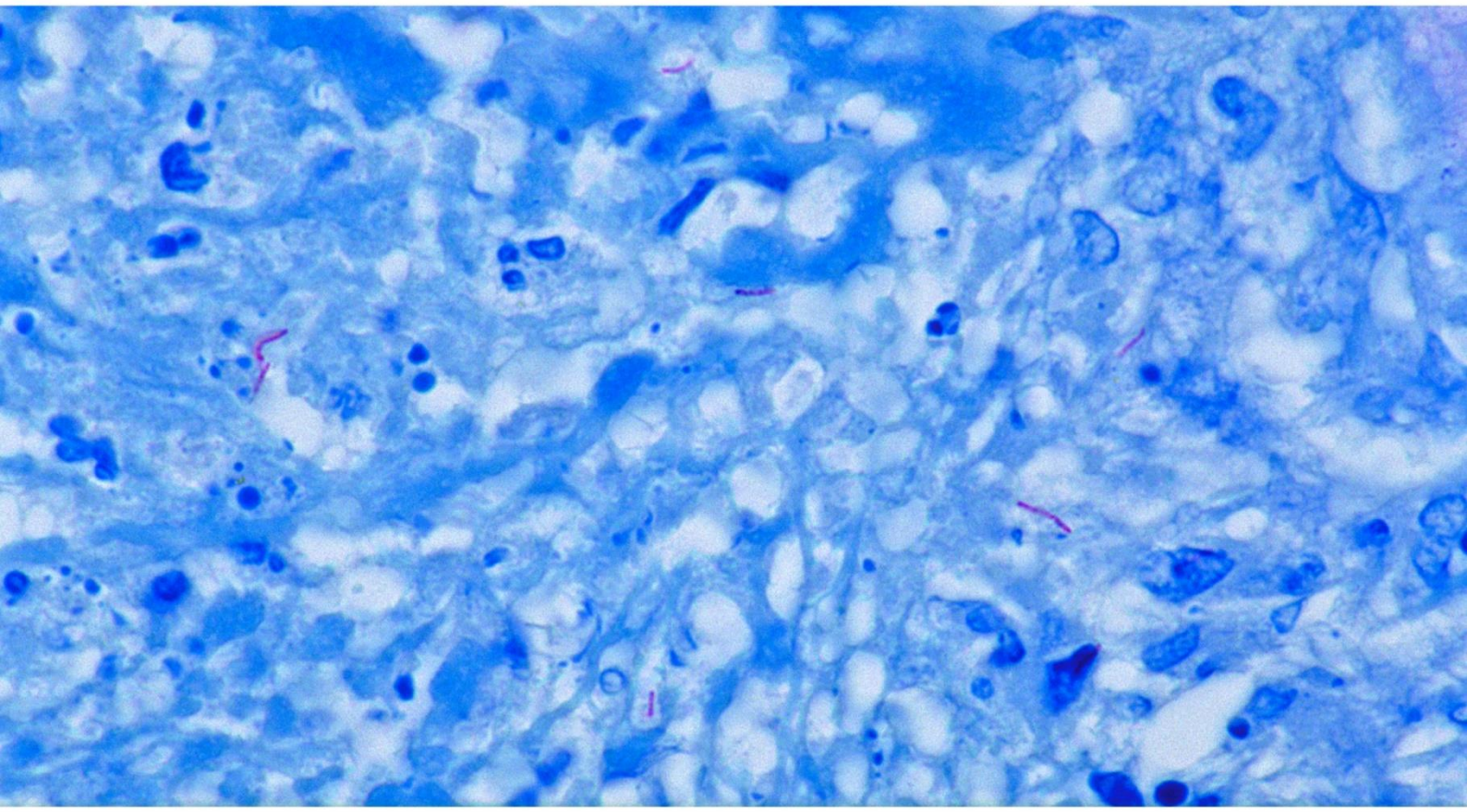
THE 2016 TB SUMMIT

Abstracts



21st - 23rd June 2016
London, UK

EuroSciCon

This annual three day event will explore new research into TB detection, treatment and vaccination as well as new development in controlling and preventing*Mycobacterium tuberculosis* Infection. With an informal academic atmosphere and international speakers, it is the perfect environment for debate and discussion.

This event has CPD accreditation

This abstract book will be finalised two weeks before the event
http://www.TBSummit2016.com
#TB2016

Table of Contents

Invited Speakers Abstracts

Tuberculosis therapeutics that inhibit bacterial sensing and resistance to host immunity

Dr. Robert Abramovitch, Michigan State University, East Lansing, United StatesThe ability of Mycobacterium tuberculosis (Mtb) to sense and adapt to host environmental cues is essential for the pathogen to establish and maintain an infection. The two-component regulatory systems (TCS) dosRST and phoPR play a role in sensing hypoxia and environmental pH and are necessary for Mtb survival in macrophage and animal infection models. Therefore, isolation of compounds that interfere with in vivo sensing and adaptation pathways may identify new therapeutics to treat tuberculosis. This study reports an integrated approach to drug development, where fluorescent reporters are used as both novel synthetic phenotypes for drug discovery and as in vivo biomarkers for drug exposure.

Intracellular growth of Mycobacterium tuberculosis; linking microbial physiology to drug development.

Dr. Yossef Av-Gay, The University of British Columbia, Vancouver, Canada

Mycobacterium tuberculosis is an obligatory human pathogen that infects, resides, and replicates in phagosomes of alveolar macrophages. Supported by the discovery of virulence proteins that are not essential for growth under in vitro growth conditions, the macrophage represents the most suitable model for studying TB pathophysiology. In addition pahgosomes isolate the bacteria from many antibiotics used for treatment of infection, and creates a challenge for anti-TB drug discovery. Here we present the development, the validation and the results of an intracellular HTS for finding new compounds able to kill M. tuberculosis replicating in human macrophages. Protocol was developed using known standard TB drugs and was validated by a pilot screen reported earlier this year (Sorrentino et. al. AAC, 2016). We used this intracellular assay to screen compounds deriving from two sets of the commercial libraries totaling 84,000 compounds and identified 562 primary hit compounds. Using data mining, hit compounds were clustered in structural-related families, resulting in 428 new chemical entities and 134 compounds belonging to families previously investigated or with known mechanism of action. Cytotoxic properties were determined in HepG2. Hits are now under further investigation for their potency in vitro, Mode of action in THP1 macrophages and for growth in defined carbon sources. The results here presented highlight a promising strategy for the identification of intracellular mycobacterial inhibitors with a potential new mechanism of action.

Innate immune recognition of pathogenic mycobacteria

Dr. Antje Blumenthal, The University of Queensland Diamantina Institute, Woolloongabba, Australia

Toll-like receptors (TLRs) are arguably the most studied and best understood pattern recognition receptors. Their contribution to the host response in mycobacterial infection is underpinned by findings in humans and experimental model systems. Radioprotective 105 kDa (RP105; CD180) is an unconventional member of the TLR family that promotes macrophage inflammatory cytokine responses and host resistance in Mycobacterium tuberculosis infection. We discovered that RP105 engages PI3K signalling to direct cytokine trafficking and compartmentalisation of intracellular signalling in mycobacteria-infected macrophages. This identified novel innate immune signalling axis that directs host responses in mycobacterial infection and first insight into the distinct molecular mechanisms by which RP105 shapes macrophage functions.

Improvements in Energetics-Based Methods for Target Identification in TB Drug Discovery

Dr. Cristiano V. Bizarro, Pontifícia Universidade Católica do Rio Grande do Sul – PUCRS, Porto Alegre, Brazil

In recent years, phenotypic screening is assuming a leading role in TB drug discovery. However, the development of new drugs from bioactive compounds obtained in screening campaings requires the identification of the cellular targets responsible for their biological activities. A new energetics-based method for target identification is presented, in which proteins incubated with or without a ligand and submitted to a brief proteolytic pulse are directly analyzed and compared using a label-free semi-quantitative mass spectrometry strategy, dispensing the SDS-PAGE readout, and greatly improving the throughput. Proof-of-principle and applications for TB drug discovery are discussed.

Integrated interdisciplinary approaches to tackle extensively drug resistance in TB

Dr Sanjib Bhakta, Institute of Structural and Molecular Biology, Birkbeck, University of London and UCL, UK

Tuberculosis (TB) has re-emerged as a serious public health threat worldwide because of an alarming increase in the mortality rates due to extensively-drug resistant Mycobacterium tuberculosis (XDR-TB) strains and a deadly liaison between HIV and M. tuberculosis infection. Development of an effective drug treatment with novel mechanisms of action is urgent to tackle antibiotic resistance in tuberculosis (TB).

An important element in intracellular survival and consequent pathogenesis of M. tuberculosis is its distinctive cell-wall, of which peptidoglycan is a major structural, functional and regulatory component. The cytoplasmic steps of the biosynthesis of peptidoglycan are catalysed by a series of ATP-dependent ligases and they play pivotal role by utilising ATP while incorporating specific amino acids sequentially to the C-terminus of the stem peptide; steps critical for trans-peptidation. They share a similar reaction mechanism and essential for the growth of M. tuberculosis. As the reactions catalysed by these enzymes provide key precursors for the cell wall biogenesis and recycling, they are therefore considered as excellent therapeutic targets at the different physiological stages of the TB pathogen.

We have characterised the structure, function, regulation and inhibition of this group of enzymes and integrate that with a whole cell chemical biology evaluation model we have developed.

Adjunctive immunotherapy of tuberculosis using vitamin D and phenylbutyrate: a randomized double-blind clinical trial

Dr. Susanna Brighenti, Center for Infectious Medicine, Karolinska Institutet, Stockholm, Sweden

Advances in drug discovery and alternative treatment strategies are urgently required for patients with chronic pulmonary TB. In a randomized, double-blind placebo controlled clinical trial conducted in Ethiopia, we investigated if a new therapeutic rationale based on treatment with vitaminD and phenylbutyrate (PBA) can be used to strengthen mucosal immunity including antimicrobial activity in macrophages through induction of the antimicrobial peptide, human cathelicidin LL-37. We aimed to explore if adjunctive immunotherapy with vitaminD/PBA could limit bacterial load and improve the response to standard chemotherapy towards a rapid clinical recovery among patients with newly diagnosed sputum-positive or sputum-negative pulmonary TB.

Genomes, structural biology and drug discovery: Understanding antimicrobial resistance in tuberculosis

Professor Tom Blundell, University of Cambridge, Cambridge, United Kingdom

Second generation methods of gene sequencing now allow us to follow the emergence of resistance as Mycobacterium tuberculosis evades the new therapeutics targeting it. I will discuss our work funded by Gates Foundation HIT-TB and EU FP7 MM4TB on tuberculosis. I will describe structure-guided, fragment-based approaches that have been successful against oncology in the company Astex that I cofounded in Cambridge and are now being used in tuberculosis drug discovery. I will discuss computational approaches that facilitate understanding mechanisms leading to emerging resistance in tuberculosis and discuss possible approaches to combating this.

3 million TB cases missing: can community based services find them?

Professor Luis E. Cuevas, Liverpool School of Tropical Medicine, Liverpool, United Kingdom

There are over 3 million cases of tuberculosis in the world that never reach the health services. The new Sustainable Development Goals include reaching all cases of tuberculosis by 2035. We need to find novel approaches that reach these missing cases to make reach this SDG. We have tested novel approaches to identify missing cases in the community in Nigeria, Ethiopia and Yemen. This talk will provide an overview of the advantages and disadvantages of these approaches and potential implications for tuberculosis control

Capturing Geographic Complexity in a Homeless "Neighborhood": spatial video geonarratives (SVG) in Skid Row, Los Angeles

Professor Andrew Curtis, GIS Health & Hazards Lab, Kent State University, Kent, United States

TB in US cities presents a considerable challenge because homeless populations, who are most at risk, also have the least amount of associated data. Spatial video (GPS-enabled cameras) can be used to map key places in the homeless landscape while simultaneously collected expert commentary from both professionals and the homeless, can help reveal the internal complexity of the homeless neighborhood. Using Skid Row, Los Angeles as a case study, these geonarratives were transcribed and interpolated onto the video coordinate stream, before being compared with other TB related static interviews to help create a better understanding of the neighborhood's health map.

Additional co-authors for the paper include:
Dr. Jacqueline Curtis, Assistant Professor of Geography, GIS Health & Hazards Lab, Kent State University
and
Charles Felix, J.D., M.P.H., Tulare County Health & Human Services Agency, Tulare, California

Understanding TB transmission with whole-genome sequencing and modelling

Dr. Caroline Colijn, Imperial College London, London, United Kingdom

I will discuss modelling and inference tools to improve understanding of TB transmission using whole-genome sequencing data. This is a rapidly-evolving field with advanced statistical tools and excellent data at hand, but considerable uncertainty remains in inferring who infected whom. Other aspects of transmission, such as the delay between becoming infected and infecting others, are more robustly estimated from data.

Plants a source of antitubercular agents

Dr. Maria del Rayo Camacho-Corona, Universidad Autónoma de Nuevo León, Nuevo Leon, Mexico

Plants have been the source of new drugs since ancient times. Our research group select nine plants used in Mexican traditional medicine to treat tuberculosis and other respiratory diseases. Thirty six extracts were prepared and tested against Mycobacterium tuberculosis H37Rv. Results showed that five extracts were the most active. From the active extracts were isolated and characterized the compounds responsible of antitubercular activity. In addition, the mode of action of two active principles was determined and 28 semi-synthetic analogues were prepared and tested against M. tuberculosis, yielding two potent compounds.

Design and synthesis of antitubercular small molecules targeting dru-resistant mycobacteria

Dr. Daniele Castagnolo, Lecturer, King's College London, Institute of Pharmaceutical Science, London, United Kingdom

A novel class of pyrrole derivatives and thioridazine analogues have been designed, synthesised and biologically evaluated against a number of mycobacteria. The novel compounds have originally been designed as hybrids of the anti-tubercular drugs BM212 and SQ109 that showed common features and similar spatial conformations. Five novel compounds showed submicromolar anti-tubercular activities on pathogenic Mycobacterium tuberculosis, and two of them proved to be highly active also against MDR-TB clinical isolates. The potent anti-tubercular derivatives also showed minimal eukaryotic cell toxicity, turning out to be an excellent lead candidates for preclinical trials.

The Mtb Divisome: Initial Structural Efforts on Drug Targets

Dr. Timothy A. Cross, Florida State University, Tallahassee, United States

Many of the proteins that comprise the cell division apparatus for M. tuberculosis (Mtb) have been identified. but analogs for other proteins that play critical roles in better-studied bacterial divisomes have yet to be identified for Mtb. Through pulll down assays and bacterial two hybrid assays many of the interacting partners in the divisome have been identified. Here, we characterize membrane protein structure and interactions in a liquid crystalline lipid bilayer environment that stabilizes the protein's native structure. I will describe our studies of CrgA, ChiZ and CwsA for the Mtb divisome.

Communicating risks and benefits of preventive tuberculosis treatment for shared decision making

Dr Claudia Dobler, University of New South Wales, Sydney, Australia

What information is important for patients and physicians to make informed decisions about preventive TB treatment? Is shared decision-making between patient and health care provider a valid approach for preventive TB treatment, or should the public health perspective guide our conversations with patients?

Development and deployment of new TB drugs

Dr. Gerry Davies, University of Liverpool, Liverpool, United Kingdom

For the first time in forty years, real changes are taking place in anti-tuberculosis therapy, holding out real hope for patients with drug-resistant disease. Advances in drug development are hampered however by uncertainty about the value of animal models, an incomplete understanding of the pharmacokinetic and pharmacodynamic determinants of success and conservatism in clinical trial design. This talk will review recent important findings in the science underlying TB treatment and the implications for improving and accelerating drug development.

Purinergic signaling in the immune response to severe tuberculosis

Professor Maria Regina D'Imperio Lima, Universidade de São Paulo, São Paulo, Brazil

Dead cells as a result of tissue damage are quickly engulfed, but before disappearing, warn the surrounding cells so that repair programs are activated. The recognition of cell damage by innate immune system contributes to development of inflammatory immune responses and tissue repair, but may also exacerbate tissue injuries. We evaluated the role of P2X7 receptor that is triggered by extracellular ATP, a damage signal released by necrotic cells, and adenosine, a byproduct of ATP, in the immune response to hypervirulent mycobacterial strains. We found that these molecules contribute to lung pathology and to suppression of CD4 T cells, respectively.

Pathway for synthesis of methylglucose lipopolysaccharides in Mycobacterium tuberculosis

Dr Nuno Empadinhas, CNC - Center for Neuroscience and Cell Biology, University of Coimbra, Portugal

The mycobacterial cell envelope mycolic acids are synthesized from fatty acids synthesized in the cytoplasm, where methylmannose polysaccharides (MMP) and acylated methylglucose lipopolysaccharides (MGLP) are also generated probably to modulate fatty acid metabolism, storage and envelope lipids assembly. Since MMP seems to be restricted to rapidly-growing mycobacteria, MGLP is likely vital for Mycobacterium tuberculosis (Mtb). We identified genes coordinating the early events of MGLP biogenesis, some of which proposed to be essential for Mtb growth, and characterized the enzymes for their drug discovery potential. The quest for new TB therapies urges comprehensive understanding of MGLP metabolism in vivo to help expose essential mechanisms worth targeting.

Transcriptional Regulation in Mycobacteria

Dr. Eric Galburt, Washington University School of Medicine, St. Louis, United States

Transcription initiation in mycobacteria including M. tuberculosis appears to be regulated via unique mechanisms when compared to E. coli. Specifically, CarD and RbpA are two transcription factors with no homologues in E. coli, but essential for M. tuberculosis. Both factors bind to the transcription initiation complex and modulate gene expression. We have recently made progress in understanding the distinct mechanisms of these factors. Although the exact roles of these factors during infection are not yet clear, this work brings us closer to understanding the possible roles played by these unique and essential molecules in the lifecycle of M. tuberculosis.

TaqMan Array Card for genotypic drug resistance testing

Professor Eric R. Houpt, University of Virginia, Virginia, United States

Phenotypic drug susceptibility testing is slow and laborious. We evaluated the accuracy a genotypic TaqMan Array Card and the MYCOTB plate in 3 countries on 212 MDR-TB isolates. The overall accuracy for 6 second-line drugs was 87% for TAC and 88% for the MYCOTB plate. Using a consensus gold-standard, the TAC, MYCOTB plate, and the conventional phenotypic method performed similarly for second line drugs, however the former methods offer speed, throughput, and quantitative

susceptibility information. The TAC method is particularly well suited to surveillance in central labs because it is fast, high throughput, and biosafe.

Diagnostic approaches in mediastinal TB

Professor Onn Min Kon, Imperial College Healthcare NHS Trust, London, United Kingdom

Mediastinal lymph node TB is an increasingly common presentation of TB and is also the potential 'portal' for dissemination of TB after primary infection. This talk will review the presentations that can be associated with this site of disease and the state of the art diagnostic approaches to this manifestation including endobronchial ultrasound sampling.

A vaccine pipeline - phage display for biomarker identification and generation of human antibodies for diagnostics and therapy

Dr. Gustavo Moreira, Technische Universität Braunschweig, Institut für Biochemie, Biotechnologie und Bioinformatik, Braunschweig, Germany

The identification of biomarkers from pathogens is a prerequisite for the development of vaccines and diagnostic assays. We are using phage display to identify immunogenic proteins using bacterial genome libraries and in parallel, we developed a human antibody generation pipeline. The combination of both phage display based technologies led to a "vaccine development pipeline".

In this presentation, the technology will be described and examples for the identification of biomarkers of different pathogens will given. In addition, the development of human recombinant antibodies for the detection of Mycobacterium tuberculosis antigen 85B will be shown.

TB persisters - resistance is futile, time to end them all

Dr. Yanmin Hu, St George's University of London, London, United Kingdom

Effective tuberculosis control is hindered by bacterial persistence, which necessitates prolonged multi-drug therapy, leading to poor patient compliance, high relapse rates and drug resistance. These persistent organisms become undetectable using the traditional microbiological methods. They failed to show acid fast staining and multiply on agar plates or in broth medium. We have identified and quantified persistent bacteria using resuscitation promoting factors (RPF). We found that RPF-dependent persisters were present in vitro and in mouse infection models. If we remove RPF-dependent persisters, treatment duration was shortened with a reduced relapse rate. RPF resuscitation could be used as a potential risk-stratification tool for the treatment of tuberculosis.

Toxin:antitoxin modules as regulators of TB growth and persistence

Associate Professor Shaleen Korch, Midwestern University, Department of Pharmacology, Glendale, Arizona, USA

One aspect of Mtb's pathogenic success is undoubtedly its ability to adapt to adverse environments encountered during infection of human macrophages. By mechanisms not fully understood, Mtb is able to transition from active growth to dormancy and can persist for extensive periods of time, with the potential of causing reactivation disease. Remarkably, Mtb encodes 90+ TA modules belonging to TA families relBE, vapBC, parDE, higBA and mazEF, suggesting involvement of toxin:antitoxin genes in Mtb pathogenesis. This talk will focus on the role of TA modules as regulators of cell growth and potential effectors of mycobacterial persistence, with an emphasis on the relBE family.

Synthesis and anti-tubercular activity of 2-nitroimidazooxazines with modification at the C-7 position

Dr. Ill Young Lee, Korea Research Institute of Chemical Technology, Daejeon, South Korea

The nitroimidazooxazine PA-824 represents a new class of bio-reductive drug to treat TB. We report a 2-nitroimidazooxazine derivative with modification at the C-7 position that exhibited better activity than PA-824 against Mycobacterium tuberculosis (Mtb) H37Rv strain in vitro. For the synthesis of 7-substituted-2-nitro-6,7-dihydro-5H-imidazo[2,1-b][1,3]oxazine instead of substituted 4-(trifluoromethoxy)benzyl ether at C-6 position in PA-824, we tried achiral and chiral synthetic pathway. Also the structure-activity relationship (SAR) of these compounds will be presented.

On the identifiability of transmission dynamic models for infectious diseases

Dr. Jarno Lintusaari, Aalto University, Espoo, Finland

Transmission dynamic models are widely used in combination with computer simulations for studying epidemiological properties of infectious diseases. Estimates of key epidemiological quantities may depend strongly on both modeling and prior assumptions in a non-obvious manner. We illustrate for tuberculosis how seemingly innocent assumptions, e.g. about the size of the infectious population, may dominate some estimates completely. On a positive note, we found that the infectious population size can actually be inferred if the remaining epidemiological parameters are already known with sufficient precision. This talk is based on recent article by Lintusaari et al., published in GENETICS, DOI: 10.1534/genetics.115.180034.

Host defense mechanisms during early stages of tuberculosis in zebrafish

Dr Annemarie H. Meijer, Institute of Biology, Leiden University, Gorlaeus Laboratory, Leiden, Netherlands

Infection of zebrafish with Mycobacterium marinum recapitulates hallmarks of human tuberculosis pathology, including the formation of granulomatous lesions. The early stages of these granulomas are uniquely accessible in the optically transparent zebrafish embryos. Granuloma formation begins with aggregation of infected macrophages and is promoted by mycobacterial virulence factors. We study the host factors that either function to control infection or that are subverted by the pathogen. From our recent work, a host-protective role emerged for the autophagy modulator DRAM1, while we found the chemokine receptor CXCR3 to be exploited by M. marinum to facilitate its dissemination during early granuloma formation.

Galina Mukamolova, University of Leicester, Leicester, United Kingdom

Mycobacterium tuberculosis (Mtb) has a remakable ability to adopt a special dormant state. Dormant mycobacteria are often undetectable by standard culture media and require addition of so called Resuscitation-promoting factor (Rpf). Rpf-dependent Mtb are generated during infection more resistant to antimicrobial treatment and may resuscitate causing an active disease. Rpf-dependent Mtb populations dominate sputum samples from TB patients and their dynamics during treatment may prediction treatment outcomes. The talk will summarise recent findings in the area of mycobacterial dormancy, resuscitation and Rpf-dependency.

PURE-TB-LAMP: Simple & Rapid screenging test for TB

Dr Yasuyoshi Mori, Eiken Chemical Co.,Ltd., Ohtawara Tochigi, Japan

The Loop-mediated isothermal amplification (LAMP) for Mycobacterium tuberculosis complex detection and its related technology named PURE (Procedure for Ultra Rapid Extraction) for simple sample processing method have been developed as a first screening molecular test for TB. As the results of multiple evaluation studies, PURE-TB-LAMP has found to be able to detect MTB in raw sputa with higher sensitivity and simpler processes than smear microscopy test, indicating the PURE-TB-LAMP can offer a realistic solution to improve capacity of case finding for TB in resource limited facilities like smear microscopy centers in developing countries. In this presentation, basic performance and its application of PURE-TB-LAMP will be demonstrated.

Extensive Drug Resistant Tuberculosis plus Linezolid Resistance: Linezolid resistance development in Lisbon, Portugal

Dr. Isabel Portugal, Faculdade de Farmácia da Universidade de Lisboa, University of Lisbon, Lisboa, Portugal

Drug resistant Mycobacterium tuberculosis remains as one of the major challenges and threats to tuberculosis (TB) control, globally. In Portugal, two phylogenetic clades have been associated with multi and extensive drug resistance (MDR)/XDR-TB and have shown an evolutionary trajectory towards total drug resistant (TDR) TB. In such cases linezolid is often used off-label to treat these infections. Here, we investigate the phylogenetic origin and molecular basis of resistance of a case of XDR-TB with resistance to linezolid.

Multiple Biomarkers for Reliable Identification of Mycobacterium Tuberculosis Complex in Broth Culture Media with Nanodiamond

Professor Wen Ping Peng, National Dong Hwa Universit, Taiwan

Molecular diagnostic methods for mycobacterium identification are expensive and time-consuming. Therefore, we adopted detonation nanodiamonds with matrix assisted laser desorption/ionization mass spectrometry to extract and analyze proteins secreted from mycobacterium tuberculosis complex (MTC) in BACTEC MGIT 960. We identified four proteins, ESAT-6 antigen, CFP-10 antigen, CFP-10 D7-F100 antigen, and VapB2 protein as reliable biomarkers to distinguish MTC from nontuberculous mycobacteria (NTM). 141 MTC and 92 NTM clinical samples were investigated with these biomarkers and sensitivity and specificity of MTC samples could reach 95.7% and 100%. No NTM biomarkers can be identified. The analysis can be finished within 1 hour.

Development of Dinitrobenzyl Oxadiazoles and Thiadiazoles as Selective Antitubercular Agents

Dr Jaroslav Roh, Charles University in Prague, Faculty of Pharmacy in Hradec Kralove, Czech Republic

The discovery and structure-activity relationships of dinitrobenzyl oxadiazoles and thiadiazoles as a new class of antituberculosis agents will be discussed. Majority of these compounds showed outstanding in vitro activity against Mycobacterium tuberculosis H37Rv and against six multidrug-resistant strains, with MIC values reaching 0.03 μM. The antimycobacterial effects of these compounds were highly specific because they showed no activity against other bacteria or fungi and exhibited low in vitro toxicity in mammalian cell lines, e.g. primary human hepatocytes. Furthermore, selected oxa- and thiadiazole tested on M.tuberculosis SS18b strain showed potency comparable to rifampicin pointing to their activity against nonreplicating mycobacteria.

IFITM family members restrict Mycobacterium tuberculosis infection

Dr Shahin Ranjbar, Harvard Medical School, USA

The interferon (IFN)-induced transmembrane (IFITM) proteins are critical mediators of the host antiviral response. We expand the role of IFITM proteins to host defense against intracellular bacterial infection by demonstrating that they restrict Mycobacterium tuberculosis (MTb) intracellular growth. Simultaneous knockdown of IFITM1, 2, and 3 by RNA interference significantly enhances MTb growth in human monocytic and alveolar/epithelial cells. MTb infection, toll-like receptor 2 and 4 ligands, and several proinflammatory cytokines, induce IFITM1-3 gene expression in human myeloid cells. IFITM3 co-localizes with early and, in particular, late MTb phagosomes, and overexpression of IFITM3 enhances endosomal acidification in MTb-infected monocytic cells.

Genetic Approaches to Facilitate TB Drug Development

Professor Dirk Schnappinger, Weill Cornell Medical College, New York, United States

Very few chemically novel agents have been approved for antibacterial chemotherapies during the last 50 years. Yet new antibacterial drugs are needed to reduce the impact on global health of an increasing number of drug-resistant infections, including highly drug resistant forms of tuberculosis. This presentation will use enzymes involved in the biotin metabolism of Mycobacterium tuberculosis to discuss how genetic approaches can be used to facilitate antimicrobial drug development.

Recent Developments in Childhood Tuberculosis

Dr. James Seddon, Imperial College London, UK

This talk will cover an update on our understanding of the burden of tuberculosis in children as well as recent studies that have evaluated new diagnostics and treatments for children with tuberculosis infection and disease

Rapid differentiation of Mycobacteria

Dr Linda Stewart, Queen's University, Belfast, Belfast, United Kingdom

The two most prevalent pathogens within the Mycobacterium tuberculosis complex (MTC) are Mycobacterium tuberculosis and M. bovis. TB caused by these two species is indistinguishable clinically or pathologically, however, differentiation is essential as M. bovis is inherently resistant to pyrazinamide, an anti-tuberculosis drug. Molecular techniques are currently the only means of differentiation. The intrinsic pathogenicity and antigenic complexity of the cell surface has confounded the selection of appropriate targets for diagnostic applications. Here we describe the development of a

novel lateral flow device, 'Rapid-bTB', which is able to differentiate M. bovis from M. tuberculosis, M. africanum, and NTM isolated from human sputa in MGITTM liquid cultures.

TB or not TB? - Metabolomics may hold the answer

Prof Du Toit Loots, North-West University, Potchefstroom, South Africa

Despite the fervent genomic and proteomic based research efforts to date, since its discovery in 1882, TB is still a major global problem, and hence new approaches are necessary to better characterize and diagnose this disease. Using a variety of LC-MS, GC-MS and NMR metabolomics based methodologies, we have investigated tuberculosis from a variety of different perspectives, for the purpose of identifying new biomarkers which better explain the mechanisms related to drug resistance, virulence, growth and host-microbe interactions/adaptations. Furthermore, these biomarkers are also showing promise for the development of improved diagnostic approaches, not only for identifying TB complex, but also for detecting drug resistant strains, distinguishing various Mycobacterium species, and predicting treatment outcome.

Next Generation Tuberculosis Diagnostics: Test and Treat

Dr. Elisa Tagliani Ph.D., Research Scientist, Emerging Bacterial Pathogens Unit, Division of Immunology, Transplantation and Infectious Diseases, IRCCS San Raffaele Scientific Institute, Milan, Italy

The development of better tests for the rapid diagnosis of TB and drug resistant TB will be critical for achieving the post-2015 TB targets set out by the World Health Organization. Next generation molecular tests need to be more sensitive to detect M. tuberculosis in paucibacillary samples and to have a high Negative Predictive Value to prevent unnecessary anti-TB treatment. In addition, increasing access to TB care for hard to reach population will require the development of tests with improved operational characteristic such as full portability and automatic connectivity.

Treatment of drug resistant tuberculosis – future prospects

Dr Prakash Udhawdas Tahiliani, Clinical Researcher, Prime Ever Ayurvedic Research Laboratories, Navsari, Gujarat, India

Tuberculosis is presently the leading infectious killer in the world. There is growing resistance to available drugs, and disease is becoming more difficult to treat. There are 4.8 million cases of MDR-TB each year.

Bacteria have learned supremably well how to react to threats to their well being. (Stephen Harrod). Development of drug resistance by bacteria is assumed to be more grave in coming years.

Unfortunately, giant pharmaceutical companies have completely given up the search for new antibiotics. Medicinal herbs should be our front line defence against disease as plants contain thousands of compounds, too complex for resistance to occur

Coughing and the transmission of tuberculosis

Dr. Richard Turner, Homerton University Hospital NHS Foundation Trust, London, United Kingdom

Cough is a common syptom of TB and is probably of key importance for the spread of infection. Until recently cough in TB had not been researched for almost 50 years. This talk will discuss the background to the topic and new developments in the field.

Assessment of terbium (III) as a probe for the detection of tuberculosis biomarkers

Dr. Thu-Hoa Tran-Thi, Centre national de la recherche scientifique, Paris, France

A detection method of nicotinic acid, a specific metabolite marker of Mycobacterium tuberculosis is studied in complex solutions containing other metabolites and in biological media such as urine, saliva and breath condensate. The method is based on the analysis of the luminescence increase of Tb3+ complexes in the presence of nicotinic acid due to the energy transfer from the excited ligand to the lanthanide ion. An analysis of the interference of other potential markers also present in breath is provided.

M. tuberculosis evolution

Frédéric J. Veyrier, INRS-Institut Armand-Frappier, Laval, Canada

Our laborary is trying to deciphering the evolution of Mycobacterium tuberculosis. We are using this knowledge to ret-hink our studies to screen for antibiotics compounds.

Understanding and intervening in HIV-tuberculosis

Dr. Robert Wilkinson University of Cape Town, Cape Town, South Africa

The commonest opportunistic infection worldwide that occurs in HIV-1 infected persons is tuberculosis (TB). HIV-1 co-infection predisposes to both infection by, and reactivation of TB, and modifies its natural history and clinical presentation. An increase in extrapulmonary disease is well-recognized, and early or subclinical TB disease characterised by very few or no symptoms is also common. Immunodiagnostic methods to ascertain TB sensitisation in HIV-1 infected persons are compromised in sensitivity. This presentation will review recent work in these areas and the translational consequences of those findings.

Day 1:

Oral Presentation Abstracts

Oral presentations will be added after the submission deadline

Day 2:

Oral Presentation Abstracts

Rv1458c: A NEW DIAGNOSTIC MARKER FOR M. TUBERCULOSIS COMPLEX IN A NOVEL DUPLEX PCR ASSAY

Kamal Shrivastava, Kushal Garima, Anshika Narang, Kausik Bhattacharya, Mridula Bose, *Mandira Varma-Basil*

Postal Address: Prof. Mandira Varma-Basil, Dept. of Microbiology, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi-110007, INDIA

Globally, mycobacteriology laboratories are adopting new molecular diagnostics that are more sensitive and specific than conventional diagnostic techniques. However, the expense of these assays impedes their use in routine diagnostic work up in peripheral regions. Hence, the search for new tools that can offer quality and affordable TB diagnostics is still ongoing.

In the present study, we explored the efficiency of *Rv1458c*, a putative ABC drug transporter specific for the *Mycobacterium tuberculosis complex* (MTBC) as a diagnostic marker for MTBC. We targeted a 190 bp region of *Rv1458c* and a 300bp region of *hsp65* gene in a novel duplex assay and compared it with a previously published PCR restriction analysis (PRA) that used restriction enzymes NruI and BamHI. The Rv1458c gene sequence was scanned for single nucleotide polymorphisms (SNPs) from genome sequences of 89 clinical isolates from India. The sequences were available through the Open Source Drug Discovery Program whole genome sequencing data. No variations were observed in the 190 bp region scanned in the 89 isolates.

A total of 426 clinical isolates were obtained from clinically suspected patients of pulmonary tuberculosis from 2012 to 2015. In addition to the clinical isolates of *Mycobacterium* sp., 14 mycobacterial and 9 non-mycobacterial reference strains were also included in the study. All the clinical isolates were subjected to niacin, nitrate reduction and semiquantitative catalase tests, followed by a duplex PCR, targeting the hsp65 gene and *Rv1458c*. Speciation of a subset of the isolates (n=50) was confirmed by sequencing. The hsp65 gene was amplified in all mycobacterial reference strains and clinical isolates. However, amplification in Rv1458c was observed only in MTBC. The duplex PCR assay using Rv1458c was concordant with the NruI/BamHI PRA when performed on culture isolates and was 100% specific and 100% sensitive when compared with sequencing. The assay could be developed to be used as a screening test to identify MTBC in clinical specimens in peripheral laboratories with frugal resources.

FIELD STUDY ON A LOW COST HIGH-THROUGHPUT BLOOD BASED DIAGNOSTIC TEST FOR TUBERCULOSIS

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Approximately, two billion people worldwide are infected with Mycobacterium tuberculosis (M. tb.), the etiologic agent of tuberculosis (TB). A tenth of the infected individuals (about 200 million) develop active TB. A majority of these active TB patients live in the resource poor developing countries. The current standard-of-practice diagnostic methods which include sputum smear (SS) microscopy, culture and X-ray, are insensitive, inefficient, cumbersome or too expensive. The most widely used test in TB endemic countries is SS microscopy (WHO standard). This test has a low sensitivity (30-50% under optimal conditions). Therefore, there is an urgent need for low cost, efficient, high-throughput and accurate diagnostic approaches. We have developed a blood based immune biomarker diagnostic system for TB diagnosis. Data from a field study will be presented. Antibodies against ten M. tb antigens were studied by microbead suspension array in active pulmonary TB patients in a TB endemic country, Pakistan. Patients with active TB, smear positive (SS+) or smear negative (SS-), and confirmed by culture, were included in the study. Disease control group comprised of chronic obstructive pulmonary disease (COPD) patients (TB and COPD clinical symptoms are similar). In SS+ and culture positive TB patients (n=98), our assay sensitivity was 94.9%. In SS- and culture positive TB patients (n=101), sensitivity was 87%. Based on the results from COPD patients (n=55), the test specificity was 96%. In conclusion, multiplex antibody test is a highly sensitive blood based test demonstrating successful detection of a majority of confirmed TB cases (91.46% overall sensitivity, and 96% specificity). Compared to sputum smear test, the multiplex antibody based blood test has an advantage not only in higher sensitivity but also in high-throughput, improved diagnostic workflow, efficiency and potential for automation. Importantly, this test is cost effective in TB endemic countries and can be performed on either blood plasma or dried blood spots (DBS) on filter paper. DBS enable easy transport of samples from even remote areas, where the healthcare infrastructure is not developed (e.g. villages, refugee camps etc.), to diagnostic test centers.

Day 3:

Oral Presentation Abstracts

Oral presentations will be added after the submission deadline

THE PROFILE OF MYCOBACTERIAL ANTIGEN-DRIVEN CYTOKINE RESPONSES AND THE EXPRESSION OF SIGNAL TRANSDUCTION RECEPTORS IN TUBERCULOSIS

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Tuberculosis (TB) remains a serious worldwide health problem, whose solution requires a better understanding of anti-tuberculosis protection. With regard to this problem, we decided to identify the parameters of the host immune system which is weakened by M. tuberculosis and allow bacteria to avoid immune surveillance leading to TB disease. Research objectives were achieved through a quantitative assessment of the 1) production of IFN-gamma, TNF-alfa and IL-10 in whole blood cultures stimulated with live attenuated M. bovis BCG or virulent M. tuberculosis bacteria, ESAT-6 antigen and purified tuberculin proteins PPD, 2) surface expression of mCD14 and TLR2 signal transduction receptors and LFA-1 integrin on monocytes 3) serum concentration of soluble sCD14 receptors, in the groups of TB patients (43), patients with nonmycobacterial lung diseases (46) and healthy controls (41). The most important results involve the following observations: 1) quantitative rather than qualitative changes in the IFN-gamma, TNF-alpha and IL-10 responses to mycobacterial antigens differentiate TB patients from healthy controls, 2) a simultaneous increase in the monocyte expression of mCD14 and LFA-1 and serum sCD14 levels, in TB patients may be a biomarker potentially useful in TB diagnosis, 3) a significant domination of TLR2high monocytes in TB patients was correlated with weakened mycobacterium antigen-driven IFN-gamma production and a downward trend in the frequency of lymphocytic infiltrates in the lung and positive skin reactivity to tuberculin. This allows us to suggest a potential contribution of TLR2 receptor to the transmission of a negative signal in active TB.

Poster Presentation Abstracts

Poster abstracts will be finalised weeks before the event

DECIPHERING THE PHYSIOLOGICAL STATE OF DRUG RESISTANT *MYCOBACTERIUM TUBERCULOSIS* STRAINS

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Rationale: The rising incidence of drug resistant *Mycobacterium tuberculosis* strains negatively influences Tuberculosis control. These strains evade the killing action of old and new anti-TB drugs, rendering current therapy ineffective and resulting in prolonged treatment. Additionally, limited data exist on the physiological changes of *M.tb* during treatment. For this reason, we aim to assess the physiological changes of *M.tb* at the transcriptional and protein level during antibiotic treatment, a consequence of TB treatment failure.

Objective: To decipher how the physiological state of drug resistant *M.tb* exposed to sub-lethal concentrations of isoniazid (INH) contributes to prolonged TB treatment.

Methods: Pan-susceptible Beijing clinical isolate (K636), K636 rifampicin resistant *in vitro* mutant and laboratory strain H37Rv were selected for characterization. To determine the growth, the strains were cultured in 7H9 enriched media and on 7H11 agar plates, for daily OD_{600} readings and CFU/ml assessment. To assess the optimal concentration of INH by a titration kill-curve, the strains were exposed to a range of INH concentrations. Additionally, total RNA was extracted and purified (after 24 hrs INH exposure), for gene expression analysis by Quantitative Real-time polymerase chain reaction (qRT-PCR) for selected genes *kasA, accD6, acpM* and *ahpC*.

Preliminary Findings: No significant difference in growth was observed between the strains assessed. Additionally, it was observed that the sub-lethal concentration of INH is 0.03 µg/ml and 0.02 µg/ml for K636 and H37Rv, respectively. qRT-PCR is an important step to validate that the selected drug concentrations are influencing mycobacterial growth as expected. Thus, significant gene expression changes (>2-fold increase/decrease) in *kasA, accD6, acpM and ahpC* was observed for the strains after 24 hrs treatment with sub-lethal concentrations of INH (0.03 µg/ml and 0.02 µg/ml).

Discussion: The variation in sub-lethal concentrations of INH observed affirms what has been shown in the literature, that H37Rv is more resistant than clinical strains. The latter suggests that the strains might employ different adaptive mechanisms to survive INH drug exposure. Additionally, the significant changes in transcriptional level of *kasA, accD6, acpM and ahpC,* validate literature reports. These findings are essential for further physiological characterization of the studied *M. tb* strains by RNA-sequencing as it is anticipated that the associated physiological changes will be reflected in their total transcriptome.

MUTATION PROFILE ASSESSMENT OF RIFAMPICIN AND ISONIAZID RESISTANT TUBERCULOSIS BY USING GENOTYPE MTBDRPLUS ASSAY DIRECTLY FROM SPUTUM SAMPLES AT TERTIARY CARE CENTRE IN INDIA.

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Background: Multidrug-resistant tuberculosis (MDR-TB) is the most worrisome element of the pandemic of antibiotic resistance. Early diagnosis of MDR-TB not only enables prompt initiation of treatment but will also curb the transmission of disease in the community.
Objective: The objective of the study was to analyse the drug resistance and mutation profile of rifampicin and isoniazid resistant Mycobacterium tuberculosis directly from clinical samples using GenoType MTBDRplus assay.

Methods: A total 634 previously treated acid-fast sputum-smear positive MDR-TB suspected patients were enrolled from January 2012 to August 2015 at the All India Institute of Medical Sciences hospital, New Delhi, India. Sputum samples from all patients were subjected to molecular GenoType MTBDRplus assay and the results were compared with conventional drug susceptibility testing (DST) by using Lowenstein-Jensen (L-J) proportion method. Mutation profile was analysed with GenoType MTBDRplus assay results.

Results: Among 513 conventional DST results, 107 (21%) were MDR-TB, 50 (10%) were resistant to isoniazid but sensitive to rifampicin; 21 (4%) were resistant to rifampicin but sensitive to isoniazid and remaining 335 (65%) were sensitive to both rifampicin and isoniazid. Sensitivity and specificity of the GenoType MTBDRplus assay were 95% and 98% for detection of rifampicin resistance; 89% and 99% for isoniazid and 96% and 99% for MDR-TB. Mutations at codon 531(Ser-Leu) of rpoB gene and codon 315 (Ser-Thr) of KatG gene were the dominant mutation for rifampicin resistant and isoniazid resistant tuberculosis respectively. Both mutations were found more commonly in MDR-TB cases. Turnaround-time of GenoType MTBDRplus assay was 2 days.

Conclusion: The genotype MTBDRplus assay is highly sensitive and specific for early detection of MDR-TB with very short turnaround time. The test can be useful for early detection of most common mutations conferring resistance to rifampicin and isoniazid resistance.

ABDOMINAL TUBERCULOSIS NEEDING EXPLORATORY LAPAROTOMY IN CHILDREN: REVIEW OF 29 CASES.

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Abstract:
Aim: The aim of this study was to review the surgical management of abdominal tuberculosis needing exploratory laparotomy in children below 12-years of age.
Patients and Methods: This is a single institution, retrospective study included children who were operated for abdominal tuberculosis. It was conducted during Jan 2000 to Dec 2015 for 16 years, at author's Department of Pediatric Surgery, Gandhi Medical College Bhopal, India.

Result: Twenty-nine children below 12-years of the age were operated for abdominal tuberculosis during the study period. They were 18 (62.06%) boys and 11 (37.93%) girls, and comprised of 12 (41.37%) children aged 1-5 years, 9 (31.03%) aged 6-10 years, and 8 (27.58%) aged 11- 12 years. Clinically three-fourth (n=22; 75.86%) children presented as an acute intestinal obstruction and remaining one fourth (n=7; 24.13%) presented as perforation peritonitis. One-third (n=10; 34.48%) of

them also had history of tuberculosis and were on anti-tubercular therapy prior to the surgical therapy for acute abdomen and were pulmonary tuberculosis in 4, intestinal / abdominal tuberculosis in 5, and tuberculosis meningitis in 1 child. In 2 of the cases family member (father) were also active cases of pulmonary tuberculosis. Operative findings in order of frequency were; plastered abdomen n=9, perforation peritonitis n=7, bowel strictures n=7, bowel adhesions n=5, and mesenteric lymphadenitis only n=1. Surgical procedures executed in order of frequency were; partial / complete adhesiolysis n=11, ileo-ileal resection and anastomosis / perforation repair n=8, exploration and temporary ileostomy n=5, stricturoplasty n=3, and ileo-colic anastomosis n=2. There were 8 (27.58%) post-operative deaths. Six of them were very sick and had poor general condition (pre-operative) and presented with bowel perforation.

Conclusions: Abdominal tuberculosis is still a challenging problem in the developing countries and children may present with an acute intestinal obstruction or perforation peritonitis and warrants an urgent surgical intervention. Surgical therapy for abdominal tuberculosis is associated with considerable mortality. Pre-operative poor general condition is one of the major factors relating to the high mortalities in children needing surgical therapy.

Key-words: Abdominal Tuberculosis; Bowel Resection; Children; Exploratory Laparotomy; Ileostomy; Intestinal Obstruction; Pediatric Surgery; Perforation Peritonitis.

CYTOKINE IMBALANCE IN BCG-PRIMED MICE TREATED WITH CYCLOPHOSPHAMIDE.

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Despite the wide use of BCG (Bacilli Calmette-Guerin) vaccine, one-third of the world population has Latent Tuberculosis Infection (LTBI). These individuals can develop active tuberculosis (TB) via endogenous reactivation of the dormant Mycobacterium tuberculosis bacteria. The risk of TB reactivation is dramatically increased in the patients undergoing immunosuppressive therapy. Although this problem is a growing issue, there is a lack of knowledge about the relationship between the immune defects caused by suppressive agents and tuberculosis bacteria in breaking the previously acquired specific adaptive immunity. In the study we compared selected cellular parameters and bacterial load in BCG-primed immunocompetent or cyclophosphamide (CTX)-immunosuppressed C57/BL6 mice. The animals were injected intradermally (i.d.) in ear skin with 10 million live BCG bacteria and next the mice were injected every day with CTX at a dose of 50 mg/kg b.w. through intraperitoneal route, and observed for 21 days. The Bio-plex Suspension Array System was used for quantification of a murine cytokine panel in serum. From the seventh day of CTX administration the escalating weight loss has been observed, after which a significant increase in peripheral blood monocyte counts was noticed. Shortly after CTX administration, ConA-driven proliferation of draining lymph node lymphocytes was significantly increased, and at the seventh day of CTX administration it was dramatically weakened below the control value. CTX administration for seven-fourteen days caused a significant inhibition of proliferation of ConA-induced spleen cells. The levels of IL-2, GM-CSF, IL-4, and particularly of IL-5, were increased in CTX-immunosuppressed mice compared to immunocompetent controls. The serum levels of IFN-gamma, IL-12 and IL-10 were comparable between CTX-treated and control mice. The CTX-driven shift in the cytokine balance was accompanied by BCG penetration through the draining lymph nodes to the lungs and spleens. These data are in support of a possible contribution of CTX-driven cytokine imbalance to poor control of mycobacteria growth in infected mice

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DEVELOPMENT AND PERFORMANCE OF THE AUTOMATED ABBOTT REALTIME MTB RIF/INH ASSAY FOR SIMULTANEOUS DETECTION OF RIFAMPICIN AND ISONIAZID DRUG RESISTANCE

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ABSTRACT:

Background: Nine million people developed Tuberculosis (TB) in 2013. Multidrug-resistant TB (MDR-TB), defined as resistance to at least Rifampicin (RIF) and Isoniazid (INH), often results in treatment failures that can lead to fatal clinical outcomes. GeneXpert MTB/RIF does not detect INH resistance, and misclassifies RIF mono-Resistance as MDR-TB. GenoTypeMTBDRplus detects RIF and INH resistance but is a tedious manual process and requires manual interpretation of results. Abbott RealTime MTB RIF/INH assay is a new automated, qualitative realtime PCR test for simultaneous detection of RIF and INH drug resistance in Mycobacterium tuberculosis Complex (MTBC) from sputum, bronchial alveolar lavage (BAL), or decontaminated NALC sediments of sputum and BAL. The rapid detection of both RIF and INH drug-resistance causing mutations could facilitate initiation of appropriate drug therapy thus reducing MDR-TB transmission.

Methods: DNAs were extracted from MTB or MTB suspect specimens using m2000sp, followed by realtime PCR detection on m2000rt (Abbott Molecular Inc.). Abbott RealTime MTB RIF/INH assay performance was evaluated by assessing the impact of potential interfering substances, dynamic range, limit of detection (LoD), reproducibility, clinical sensitivity and specificity of over 200 MTB patient specimens as determined by standard Drug Susceptibility Testing (DST).

Results: Abbott RealTime MTB RIF/INH assay was not impacted by 13 potential interfering substances, had a dynamic range of 103 to 108 copies/mL , an LoD claim of 60 CFU/mL, reproducibility of 100.0% (159/159) at 3x LoD, RIF sensitivity of 94.8% (91/96) and specificity of 100% (120/120), and INH sensitivity of 88.3% (83/94) and specificity of 94.3% (116/123) that were similar to the comparators' results (GeneXpert MTB/RIF for RIF and GenoTypeMTBDRplus for INH) of the same sample population.

Conclusions: Abbott RealTime MTB RIF/INH Assay demonstrated robust performance with reproducible and reliable results.

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COMPUTATIONAL STUDY OF MTB DNA MAKING NUCLEOTIDE SYNTHESIS REACTION

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TB is one of the top ranking infectious diseases. Existing drugs for TB are becoming ineffective for the Drug resistant bacteria. So it necessiates the need to understand the basic action of bacteria by studying how it makes it's DNA. Many studies reported the mechanism of a few reactions making the nucleotide meant for the synthesis of bacteria's DNA. In our study we devoted to understand electronically the reaction between Phosphorribosyl pyro phosphate and Orotate to give orotidine mono phosphate using orotate phosphoribosyl transferase as an enzyme and magnesium ion as a cofactor. We have studied the presence of different metal ions as the activators or inhibitors for the reaction. We propose if the activator metal ion can be replaced with the inhibitor meatl ions the DNA synthesis can be hindered. We also developed kinetic models to understand the synthesis and consumption of nucleotide, orotidine mono phosphate. We also carrying studies using orotate analogue inhibitors there by inhibiting the nucleotide synthesis. Our whole study tried to understand

the basic interactions at the electronic level there by contributing to the different chances of hindering nucleotide synthesis which may hinder the progress of DNA making.

DIETARY VITAMINS AND CAROTENOIDS IN RELATION TO RISK OF ACTIVE TUBERCULOSIS IN A PROSPECTIVE COHORT STUDY

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Rationale: Tuberculosis (TB) infection is characterized by increased levels of oxidative stress, but the potential role that dietary antioxidants may play in preventing active disease has not been examined.

Objectives: We aimed to investigate the association between dietary intake of antioxidant vitamins (A, C, and E) and carotenoids (α-carotene, β-carotene, β-cryptoxanthine, lycopene, and lutein) and risk of developing active TB in a prospective population-based cohort in Singapore.

Methods: We analyzed data from the Singapore Chinese Health Study, which included 63,257 Chinese men and women aged 45-74 years recruited between 1993 and 1998. Dietary intake of vitamins and carotenoids were determined using a validated semi-quantitative food frequency questionnaire. Incident cases of active TB were identified via linkage with the nationwide TB registry up to 31 December 2014. The relation between dietary vitamins and carotenoids and risk of active TB was estimated using Cox proportional hazards models with adjustment for other potential risk factors of TB infection.

Results: We identified 1,186 incident cases of active TB over a mean follow-up of 9.1 years. Increased dietary intakes of vitamins, A, C, E were associated with reduced risk of active TB. Compared to the lowest quartile intake, the hazard ratio (HR) [95% confidence interval (CI)] was 0.79 (0.66-0.95) for the highest quartile intake of vitamin A, 0.91 (0.76-1.08) for vitamin C, and 0.86 (0.69-1.08) for vitamin E. Stratification by smoking status revealed that the reduction in TB risk with increased intake of dietary vitamins was more apparent and statistically significant among current smokers than non-current smokers; *P* for interaction between smoking status (curent, non-current) and dietary vitamins A, C, and E on risk of active TB was 0.08, 0.05, and 0.008 respectively. Compared to the lowest quartile, highest quartile intake of β-carotene was associated with reduced risk of active TB (HR 0.73, 95% CI, 0.61-0.87; *P* for trend = 0.003), and this association was significant in both current and non-current smokers (*P* for interaction = 0.24). There were no significant associations between intakes of other carotenoids and risk of active TB.

Conclusions: Higher intake of antioxidant vitamins A, C, and E may reduce risk of active TB by ameliorating oxidative stress levels among current smokers. The reduced risk with β-carotene in both current and non-current smokers suggest additional effects via other anti-TB mechanisms on top of its antioxidant properties.

PUBLIC PRIVATE MIX MODEL FOR IMPROVED TB CONTROL IN LAHORE, PAKISTAN; AN ASSESSMENT OF ITS IMPACT ON CASE DETECTION & REFERRAL PROCESS

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ABSTRACT:

Objective: To evaluate the effectiveness of interventions by private pharmacies and medical stores to increase case detection through referrals of tuberculosis (TB) suspected clients in Lahore, Pakistan and to assess the problems related to the referring of the patients.

Methods: The first phase of the study was to evaluate the impact of public private mix on TB case detection. In this phase 137 Pharmacies and medical stores from different locations of Lahore were contacted for this study. Patients having apparent TB symptoms or chronic cough for more than two weeks were referred for TB case detection to the nearby National TB Control Program (NTP) centre. Referral slips were collected weekly on every visit and updated on the online tracking sheets. The patients were monitored and followed up to analyze the results of the diagnostic tests. In the second phase, a survey was conducted of all the pharmacies concerning problems that occurred during the referral of patients for TB diagnostic test.

Results: A total of 210 TB suspects were referred for tests out of which 148 (70.5%) actually appeared for the TB examination tests and 62 (29.5%) were missing and could not be followed up because their contact numbers were either wrong or switched off permanently. Forty two of 148 (28.3%) were diagnosed with smear positive TB. Out of 148, 106 (71.62%) suspects were found to be negative. As a result of second phase of the study, different problems were noted, faced by pharmacies and medical stores. People were not willing to be referred because of different reasons including lack of time (40.9 %), fear of tuberculosis (65.7 %), preferred self medication (61.3 %) , preference to family physician over public sector programs (67.9 %) and privacy issues or social stigma (52.6 %) and lack of trust on government programs (43.8 %) . The reasons of the pharmacies and medical stores for not referring patients were lack of time (65%), fear of losing customer to other pharmacy (42.3 %) & distrust in government sector programs (48.9).

Conclusion: The Public private mix program in a country like Pakistan is necessary where most of the people prefer to visit a pharmacy instead of taking appointment from a doctor. This study concludes that if the concerns of the patients and the pharmacists are sorted out and if better education and awareness is communicated to majority of the pharmacies and population, this program can be very effective towards increased early case detection. In order to make it successful on a large scale, all the private and public sectors should be involved and government's stewardship is required.

CLONALITY AND GENETIC PROFILES OF DRUG RESISTANT MYCOBACTERIUM TUBERCULOSIS IN THE EASTERN CAPE PROVINCE, SOUTH AFRICA

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ABSTRACT
Objective: To investigate the diversity of drug-resistant Mycobacterium tuberculosis isolates in the Eastern Cape Province of South Africa using spoligotyping and mycobacterial interspersed repetitive-unit-variable number tandem repeat (MIRU-VNTR) typing.

Design: Investigation of M. tuberculosis using MIRU-VNTR and evaluation of M. tuberculosis families using spoligotyping.

Results: Spoligotyping grouped 91% of the isolates into 7 clusters, while 9% of the DNA from TB isolates were unclustered from a total of 154 used. Previously described shared types were observed in 89.6% of the isolates, with the Beijing family, SIT1, actuality the principal genotype in the province, while the families T, SIT53 and X1, SIT1329 were the least detected genotype. MIRU-VNTR grouped 81% of the isolates in 23 clusters while 19% were unclustered. A combination of the VNTR and spoligotyping grouped 79% of the isolates into 23 clusters with 21% unclustered.

Conclusion: The low level of diversity and the clonal spread of drug-resistant M. tuberculosis isolates advocate that the spread of TB in this study may be instigated by the clonal spread of Beijing genotype. This research embodies imperative contribution in the lack of TB control and distribution of MTB strain types in the Eastern Cape Province.

EVALUATING A NOVEL METHOD OF DRUG SUSCEPTIBILITY TESTING AMONG SUSPECTED TUBERCULOSIS PATIENTS IN A TERTIARY CARE CENTRE IN SOUTH INDIA

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Background
Tuberculosis (TB) caused by Mycobacterium tuberculosis (MTb) remains a major global health problem. The Emergence of Multi Drug Resistant (MDR) and Extensively Drug Resistant (XDR) TB has further threatened the Global TB Control. The primary objective of the present study was to compare the results from the Reference Standard- Drug susceptibility testing (DST) using MGIT 960 to a novel method, the Index Test-combining, the first line drugs-INH and Rifampicin in a single tube of MGIT for drug sensitivity. The Index test if proven concordant may be a cost efficient technique for drug susceptibility testing.

Methods
Sputum samples were collected from 1000 suspected TB patient- both outpatient and inpatient in a Tertiary care Teaching Hospital. After digestion and decontamination, samples were stained for microscopy and cultured in liquid (MGIT) and Löwenstein Jensen (LJ) medium to isolate M. tuberculosis. Drug susceptibility testing (DST) was done against the first line (INH, rifampicin) drugs by the conventional technique, which served as a Reference Standard and compared with the Index Test- with MGIT mixing the INH and Rifampicin in the same tube.

Results
Among the 1000 samples, 202 were smear positives and 195 were identified as MTb either by MGIT or LJ medium. Of which 120 (61.5%) were sensitive strains (INH and Rifampicin). MDR strains accounted for 39 (20.0%) overall. Thirty two (16.4%) strains were INH resistant and 4(2.0%) were rifampicin resistant.194 out of 195 samples showed concordant results with both the DST techniques. The sensitivity and specificity were 99.9% and 97.4 respectively and the Kappa value was 0.966.

Conclusion
India being a high burden country for tuberculosis is in need of introducing a cost effective method for DST and rapid diagnostic methods more than any other country. This study showed that the combination of first line drugs in a single tube could be an economical method for DST, in countries where there is limited funds allocated for Tuberculosis Control. Further studies with a formal sample size will need to be planned to validate the concordance between the tests.

NEW ASPECTS OF TUBERCULOSIS IN PEOPLE LIVING WITH HIV: REPORT OF A COHORT OF 92 CASES.

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Introduction The objective of this study is to raise the issue of the resurgence of TB in People living with HIV supported in a referent center in western Algeria, its epidemiological, clinical, biological and radiological new trends, it was a prospective study during 36 months from the 01st /01/2013 to 31st /12/2015 in identifying and analyzing cases of TB / HIV co infection.

Results Ninety two cases of co-infection TB / HIV, among 880 of new cases of HIV infections studied; prevalence of (10.45%), 78 cases (78.26%) with pulmonary tuberculosis associated to extra-pulmonary and 10 cases (10.86%) strictly pulmonary tuberculosis, only 03 cases (03.26%), presented strictly extra-pulmonary TB. The sex ratio M / F = 1.08, the modal class of the age group is between 31-40 years, the fever was 38.5 ° C in 43 cases (46.73%). The diagnosis was confirmed in 36 patients (39.13%), 24 of them (26.08%) by smear, serous localization was common: 43 cases (46.73%); Pleural 11 (11.95%), pericarditis 7 (7.60%), ascites 15 (16.30%), meningitis10 (10.86%). Deep abdominal lymphadenopathy mostly 32 (34.78%), intestinal damage was present in 5 cases (6%), and bone disease 2 cases, tuberculoma of the spinal cone 1 case. Biologically: a predominance of pancytopenia and anemia with respectively 24 cases (26.08%) and 23 cases (25%), anemia 11 cases (11.95%), leukocytosis was rare only 2 cases (2.17%), the elevation of hepatic transaminases was common 20 cases (21.73%) Radiologically: radiological images were dominated by miliary in 32 cases (34.78%) associated with a cave in 2 (2,17%) and pleurisy 3 cases (3.26%).Evolution: antibacillary favorable under treatment with a mortality rate of 11.39%

Conclusion. Co-infection TB / HIV reemerged in recent years our patients taking a more invasive appearance with frequency and multifocal forms of liver disease thus causing relatively a high mortality.

THE RESURGENCE OF TUBERCULOSIS CO-INFECTION / HIV IN CHILDREN TRACKED SUPPORT PV REFERENCE CENTER FOR HIV IN WESTERN ALGERIA: ABOUT 52 CAS.

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Introduction: Tuberculosis is one of the main factors of death among PLHIV, this co-infection has dramatically re-emerged in recent years in our climate. It clothed highly polymorphic aspects; therefore its diagnostic and therapeutic will be very difficult mainly in children, the objective of this study is to describe epidemiological, clinical and progressive characteristics of this pathology in pediatrics. Materials and methods : it is a retrospective and prospective study of 198 cases of children infected by HIV and followed in the department of infectious diseases of Oran during the periode between the 01st/01/2013 and 31st/12/2015, we have identified all cases of TB / HIV.

Results: Fifty two cases of HIV / TB co-infection were collected representing prevalence (20%), the sexe- ratio is 1.5, regarding the age at which 22 cases (42%) were infants and 25 cases (48%) for children older than 5 years. Clinically the majority of children had prolonged fever associated with a cough. Regarding the type of tuberculosis: miliary was found in 17 cases (34%), the only lung injury in 7 cases (15%), Pleuropulmonary 5 patients (10%) and multifocal tuberculosis in 26 cases (50%). Hepatosplenomegaly in only 2 cases, hepatomegaly in 5 cases (10%) in splenomegaly (25%), the lymph nodes 26 (25%) 7 ascites (15%), pericarditis 5 (10%) brain tuberculoma 5 cases (10%), tubers of Bouchut 2 (4%), adrenal 1 case. Anemia was frequent (40%), side effects were dominated by Cytolysis liver, skin reactions 15%. The evolution was marked by a high rate of relapse and mortality was equal to (32%)

Conclusion: In our cohort TB was very common as an opportunistic infection and can even be underestimated, the high rate of death is may be due to late diagnosis and difficulty of the diagnostic.

ASSOCIATION OF TUBERCULOSIS SPINAL CONE AND TEN OTHER LOCATIONS DURING AN IMMUNE RESTORATION IN PATIENTS HIV +

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The medullary cone tuberculoma is a rare localization of tuberculosis, its association with other sites is rare, and its presence in concomitant with ten other visceral in an HIV + is never described in the literature, the objectives of this work is to describe a unique case of a double tuberculomas association of the terminal cone ten other locations. Materials and methods presentation of an original TB cases multiple locations associated with the medullary cone Tuberculomas. Results patient KB aged 33 recently diagnosed HIV + on antiretroviral he consulted 02 months later for paraplegia associated with febrile respiratory distress. The examination found a gradual onset over a week by muscle weakness and a facility 03 days prior to admission of paraplegia, urinary retention and polypnea. Examination showed a T Clinque to 39 ° C, confusion, delirium, dysarthria, paraplegia, and sphincter disorders. Biologically the patient had pancytopenia hyponatremia to 118 mEq / l falling within an inappropriate secretion of ADH, radiologically the thoraco-abdominal CT scan showed a typical miliary, the mediastinal lymph nodes, intra and retroperitoneal necrotic spleen multimicronodulaires, hepatomegaly, a nodule of the pancreas, the cerebral scan multiple cerebral tuberculoma and the spine MRI revealed two spinal cord injuries that measure 10mm and 7mm respectively at the patient end cone also present chylous ascites, evolution was spectacular on TB with good clinical-biological evolution Conclusion immune restoration in its infectious form, may reveal a latent tuberculosis previously, and thus lead to a spread of the infection enjendrant localistaion s multiple exceeding 10 locations as in our case; hence the rule to detect latent or overt tuberculosis before any initiation of HAART traitemnt, especially in an area endemic for TB.

ANALOGUES OF NATURAL PRODUCT DISULPHIDES FROM ALLIUM STIPITATUM DEMONSTRATE ANTI-TUBERCULAR ACTIVITIES THROUGH DRUG EFFLUX PUMP AND BIOFILM INHIBITION

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Keywords: Mycobacterium, drug efflux, biofilms, antimicrobial resistance

Introduction: Multi-drug-resistance in Mycobacterium tuberculosis is a global challenge and new molecules with pleiotropic modes of action are urgently required to tackle this menace. Natural product disulphides were isolated from the bulbs of Allium stipitatum, genus Allium, with common members like garlic, onion, leeks and chives. The objective of this research was to synthetize a series of analogues based on the naturally isolated compounds and evaluate their antibacterial activities against a panel of mycobacteria, Gram positive and negative bacteria as well as fungal strains. Their efflux pump and biofilm inhibitory effects were also evaluated because drug efflux is a well-established mechanism that contributes to antibiotic resistance in mycobacteria and the identification of efflux pump inhibitors is an attractive target in antimicrobial therapy. Inhibition of efflux pumps also leads to inhibition of biofilm formation another major contributor to antimicrobial resistance.

Method: A number of whole-cell phenotypic bioassays including spot culture growth inhibition assay, drug efflux and biofilm assays which interrogates all the endogenous drug targets simultaneously in specific physiological contexts, were used along with appropriate positive and negative controls.

Results: The synthesized methyldisulphides showed antimycobacterial activities at clinically-relevant concentrations when tested against M. aurum, M. bovis BCG, M. tuberculosis H37Rv and multi-drug resistant strains of M. tuberculosis-clinical isolates. In addition, the synthesized compounds inhibited mycobacterial drug efflux and biofilm mechanisms.

Conclusion: This study suggests that synthesized methyldisulphides are novel chemical scaffolds that have potential as templates for the design of new drugs against tuberculosis. The inhibition of efflux pumps and biofilms by these compounds is promising as it would be a way to improve the efficacy and/or extend the clinical utility of existing antibiotics.

THE CELL ENVELOPE SKELETON OF MYCOBACTERIUM TUBERCULOSIS

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Cell envelopes of mycobacteria and related taxa are characterised by the presence of a complex mycoloyl-arabinogalactan linked to peptidoglycan. Taking into account the nature of the C70-C90 long-chain mycolic acids, a proposal was made to locate mycolates as a covalently-bound inner leaflet of a mycobacterial outer membrane (MOM) (Minnikin, In: Ratledge and Stanford eds. The Biology of the Mycobacteria. London, Academic Press; 1982 pp. 95-184). The MOM of Mycobacterium tuberculosis was completed by an external layer of characteristic free lipids, such as phthiocerol dimycocerosate waxes. The discovery of additional free lipids, such as di- and pentaacyl trehaloses,

produced an updated cell envelope model (Minnikin et al., Chemistry and Biology 2002 9:545-553). Selective dye uptake confirmed the presence of mycobacterial inner (MIM) and outer (MOM) membranes (Christensen et al., Molecular Microbiology 1999 31:1561–1572) and cryo-electron microscopy allowed dimensional estimates to be made (Zuber et al., Journal of Bacteriology 2008 190:5672-5680). Very recently the highly-specialised nature of the MIM was established (Bansal-Mutalik et al., PNAS 2014 111:4958-4963). A synthesis of all these recent data enabled the realisation of a dimensionally-faithful representation of the M. tuberculosis cell envelope (Minnikin et al., In: Ribón ed. Tuberculosis–Expanding Knowledge. Rijeka: InTech-Open 2015 pp. 145-175).

EXCAVATING THE HUMAN AND MEGAFAUNAL ORIGINS OF TUBERCLE BACILLI

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A developing phylogenetic evolutionary scenario for tuberculosis involves transforming environmental Mycobacterium kansasii, via "Mycobacterium canettii", to the modern Mycobacterium tuberculosis complex (Minnikin et al., Tuberculosis 2015 95:S133-S139). Genomic and lipid biomarkers indicate minimal evidence for the presence of Pleistocene tuberculosis in Homo sapiens, the oldest being ~9ka BP from Atlit-Yam (Israel) (Hershkovitz et al. 2008 PLoS ONE 3:e3426) and ~10-11ka Syrian bones (Baker et al., Tuberculosis 2015 95:S4-S12). Widespread dissemination of tuberculosis in Pleistocene megafauna is evident, as in a 17ka BP bison (Lee et al., PLoS ONE 2012 7:e41923) and unpublished 12ka mastodons (Hiscock Site, NY) and a 40ka bison (Kent's Cavern). Pleistocene megafaunal tuberculosis, back to ~120ka, is shown by the presence of characteristic lesions, "undermining the articular surface" of metapodials (Rothschild and Martin, Naturwissenschaften 2006 93:565-569). Our developing hypothesis is that ancestral tubercle bacilli evolved principally in Pleistocene megafauna as a non-lethal "antibiosis". Thinly spread human hunter-gatherers (Stewart and Stringer, Science 2012 335:1317-1321) may have contracted tuberculosis from infected megafauna, but they were not prime evolutionary vehicles. After an evolutionary bottleneck, newly transformed virulent tubercle bacilli became particularly prominent in settled Holocene communities of Homo sapiens and the modern M. tuberculosis complex became established.

A SIMPLIFIED SCOPING REVIEW OF CONTROL STRATEGIES OF LATENT TUBERCULOSIS IN INTERMEDIATE BURDEN ASIAN COUNTRIES

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Abstract
Background: Countries with intermediate burden of tuberculosis (TB) seldom capture the attention from health care policy makers in providing tailor-made guideline for combating tuberculosis, as compare with their high or low burden counterparts. These countries are often characterized as having good medical infrastructure, yet stagnating trend of TB incidence over years. High coverage of Bacillus Calmette–Guérin (BCG) vaccine and effective use of DOTS strategy are typical strategic response to their epidemiological characteristics. Endogenous reactivation is found to be less affected by such strategies, however, and latent TB infections (LTBI) become a critical issue in TB combat effort. In response to the End TB Strategy proposed by WHO, guidelines for intermediate burden countries in latent TB control is urgently needed.

Method: A scoping review has been carried out to summarize and disseminate the research findings on LTBI control in intermediate burden countries in Western Pacific Region.. Journal articles of primary studies and reviews published in English are included in this review. We included articles focus on LTBI management or control strategies, including prevention, treatment, diagnosis, screening policy, and cost-effectiveness of such programs, in the aforementioned countries. Exclusion criteria for this review were 1) articles published before 2006, 2) articles focus on active TB, difference in

immunological response between latent and active TB, or impact of LTBI on the progression of other diseases. Keywords included latent tuberculosis, prevention, therapy, treatment, screening, diagnosis, control, management, cost, or effect. Databases searched were MEDLINE, EMBASE, Global Health, and PsyINFO.

Findings: There were 185 articles included in the review. Several themes emerged: The major theme of the research activities is the diagnostic and screening strategies (n=113). Another main focus concerns the identification of high-risk population (n=51). Only 16 of the included articles focus on the LTBI treatment and its side-effect. Five of the articles are review or evaluation on general LTBI management program(s).

Studies have highlighted the inadequacy in solely rely on either tuberculin skin test (TST) or Interferon-gamma release assay (IGRA) in diagnosing both immunocompetent and high-risk population, while some studies revealed using IGRA as a confirmatory test can be a viable option , yet more evidence would need to support its implementation. Besides close contacts, HIV positive, and health care workers, attention has been increasingly drawn to other high-risk populations such as patients undergoing anti-tumor necrosis factor (TNF) therapy, evacuating residents after major disasters, prisoners, and homeless people.

Conclusions:
This review demonstrated some unique features of intermediate-burden countries in the appropriate diagnostic tools or screening strategies to use. This shed lights on the major concern in these countries. With more evidence and systematic aggregation of data, establishing a guideline on LTBI management for intermediate-burden countries can be a potent step in achieving the End TB goal.

RV2672 METABOLIZES HOST-DERIVED FATTY ACIDS AND PROMOTES SURVIVAL OF MYCOBACTERIUM TUBERCULOSIS UNDER HYPOXIC CONDITIONS OF MACROPHAGES

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Mycobacterium tuberculosis (Mtb) has evolved strategies to persist latently inside host macrophages, only to resurrect after the host immune system weakens. Under hypoxic conditions of the quiescent phase, Mtb mainly utilizes macrophage-derived fatty acids, particularly Triacylglyecrol (TAG) molecules as nutrient source. However, little is known about the factor(s) involved in the hydrolysis and uptake of these lipid molecules. In this study, we have delineated the molecular and functional significance of a previously uncharacterized Mtb enzyme encoded by Rv2672. Our study shows that Rv2672 exhibits both protease and lipase activities in vitro. Further clarification of its lipolytic activity came from experiments that involved processing of host-derived TAGs. Evaluation of subcellular localisation demonstrated membrane-association and secretion into culture filtrate. Furthermore, in-vitro and ex-vivo models have revealed up-regulation of the protein under conditions of hypoxic stress. Knockdown of Rv2672 resulted in diminished bacterial propagation specifically in lipid-loaded foamy macrophages that mimic the persistent phase. This study provides molecular insights into nutrient utilisation from the host, thereby augmenting the current understanding of quiescence in Mtb. Such molecules secreted in latency period can be targeted as a vaccine candidate for immunisation against latent Mtb.

CONTRIBUTIONS TO TUBERCULOSIS CLINICAL DEVELOPMENT AT THE DIVISION OF MICRIOBIOLOGY AND INFECTIOUS DISEASES, NIAID

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Abstract: DMID supports extramural clinical research to improve diagnosis, treatment and prevention of diseases caused by a variety of human infectious agents.

- Since 2007, DMID has invested approximately $253 million per year to support clinical research on 189 pathogens, including tuberculosis, malaria and antimicrobial resistance
- Of the 745 unique awards, 49 percent include an international component

Major DMID Clinical Program Supporting TB

1. DMID's Vaccine and Treatment Evaluation Units (VTEUs) conduct a broad range of studies as a service to the community including Phase I, II, III, and IV clinical trials to support product development and conduct targeted research.
2. Phase I Clinical Trial Units for Therapeutics

Contract resource to assess the safety of investigational drug candidates for use against a broad range of emerging and re-emerging infectious diseases caused by viruses other than HIV as well as bacterial, parasitic and fungal pathogens.

3. Tuberculosis Clinical Diagnostics Research Consortium

CONCLUSION: The DMID clinical research program and clinical resources have contributed to the development of several drug, vaccine, and diagnostic products. Success stories include contributions to the development the TB drug candidates SQ109 and PA-824. DMID actively seeks academic, NGO, and industrial partners with innovative concepts and promising candidates for collaborative clinical development.

THE STRATEGY AND INTERVENTION OF TUBERCULOSIS CONTROL AND PREVENTION IN ELDERLY IN INTERMEDIATE BURDEN COUNTRIES: A SCOPING REVIEW TOWARDS WHO GLOBAL END TB TARGETS

PH Chung, J Li, Cyrus LK Leung, Emily Chan, EK Yeoh

Background

Tuberculosis (TB) is increasingly becoming an important problem among elderly worldwide. In despite of a comparatively high TB burden and specific risk factors, the strategic guidelines or frameworks for elderly were not well explored comparing to other vulnerable population affected by TB. Hong Kong, an intermediate burden area, has the highest TB burden in elderly. In order to comply to the WHO Global End TB targets, this study aims to examine the strategy and intervention of TB control and prevention in elderly.

Methods

A scoping review was conducted base on Arksey and O'Malley's framework. Three health literature databases (Embase, Medline, Global health) and EBM reviews were searched for original and review articles, as well as policy papers published in English between January 1990 and December 2015. Search strategy was established in a standardized procedure. Eligible articles were screened and analyzed independently by two research teams with consensus on final article inclusion.

Results

Eighteen articles met the inclusion criteria. Most of them were review articles, issued in developed countries and after 2000. Infection control, screening and preventative therapy of latent TB infection (LTBI), active case finding, initial empirical and adequate follow-up treatment with close monitoring, education and assessment were mainly identified as targeted strategy or intervention. Affecting factors or strategical obstacles comprised atypical presentation, comorbidities, delayed diagnosis, drug interaction and adverse effects in elderly patients, cost-effectiveness of case finding strategies, limitation of LTBI screening and treatment, as well as elderly in long term care facilities.

Conclusions
Current TB strategies for elderly, mostly in developed countries, highlighted the prevention of transmission, early detection, preventive and enhanced treatment, and programmatic management. Specific factors or obstacles should be taken into account for strategic improvement. However, effective strategy and interventions in elderly are still lack of evaluated evidence with rigorous epidemiological study design. Further targeted research is highly needed for global and regional policy development.

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FROM LOGIC MODEL TO SYSTEM DYNAMICS – AN INNOVATIVE APPROACH FOR THE EVALUATION OF HEALTH CARE MANPOWER RESOURCES IN HONG KONG

PH Chung, J Li, Cyrus LK Leung, Emily Chan, EK Yeoh

BACKGROUND
Manpower calculation in the healthcare fields in Hong Kong is a chronic problem. The supply/demand of manpower depends on many factors (e.g. economic environment, immigration/emigration, non-local supply, changing fields). Traditional approach for assessment usually takes a long time. The lag time from assessment to implementation for manpower planning strategies may eventually turn a shortage problem into a surplus issue (or vice versa). An innovative approach should be developed to adapt for the changing environment in a timely manner.

CONCEPTUAL FRAMEWORK
System dynamics is an approach to understanding the behavior of complex over time. It offers "simulation technique for modeling business and social systems," which deals with internal feedback loops and time delays that affect the behavior of the entire system. The use of feedback loops and stocks and flows are the foundation tools. It should be perfect for manpower planning. The crux is to develop a valid compartmental model first.

Logic model should suit the purpose. It is a graphical depiction of the logical relationships between the resources, activities, outputs and outcomes of a program. The underlying purpose of constructing a logic model is to assess the "if-then" (causal) relationships between the elements of the program; if the resources can however be used during planning and implementation.

METHOD
The project is performed from January to March 2016. The logic model for all the key demand and supply factors for healthcare manpower in Hong Kong was built, based on a consultation stage with key stakeholders to add additional references and valuable insights. With the logic model developed, system dynamics can then be applied, using data from the Census and Statistic Department, Department of Health, Medical Council and Hospital Authority. We use the data and logic to assess whether the demand of healthcare professionals (medical doctors/ nurses/ physiotherapists/ occupational therapists/ optometrists) meet the supply in the coming thirty years.

RESULT
With the ageing pattern in Hong Kong and assuming the current service standard can be maintained, there is a need to increase 20-40% of manpower in various healthcare professionals in Hong Kong by 2026. The shortage problem will be alleviated afterwards.

PUBLIC HEALTH SIGNIFICIANCE
In order to translate research into practical use, the study has to be presented in an understandable way. This innovative approach will illustrate the complex system dynamics in a easily comprehensible way. Validity of the model is supported from the key stakeholders' participation in the consultation stage.

FROM SCOPING REVIEW TO A LOGIC MODEL: IDENTIFYING THE KEY INTERVENTIONS TO ACHIEVE THE POST-2015 WHO TARGETS TO END TUBERCULOSIS

Pui Hong Chung, EK Yeoh , Cyrus Leung , Jun Li

BACKGROUND

WHO has set an END-TB target to decrease 95% of TB cases by 2035. Many areas have been improving. However, new TB cases in some intermediate burden areas (e.g. Hong Kong) was stagant in the past 10 years. If there is no co-ordinated effort, the WHO target will not be met.

CONCEPTUAL FRAMEWORK

Individual RCT or isolated mathematical modelling cannot solve the issue. Scoping review and logic model is the best way to suggest an evidence-based proposal for government to implement.

Scoping review involves the synthesis and analysis of a wide range of research and non-research material to provide greater conceptual clarity about a specific topic. It is intended to guide more focused lines of research and development. It is particular useful to provide an overview (breadth) of evidence. The key steps proposed by Arksey and O'Malley (2005) in the scoping review protocol will be used. Different stages of work include: (1) a systematic review of defined topics and identification of the priorities; (2) charting the data using narrative methods; (3) collate the results using a framework approach; (4) consultation stage with key stakeholders to add additional references and valuable insights.

Logic model is a graphical depiction of the logical relationships between the resources, activities, outputs and outcomes of a program. While there are many ways in which logic models can be presented, the underlying purpose of constructing a logic model is to assess the "if-then" (causal) relationships between the elements of the program; if the resources can however be used during planning and implementation.

METHOD

The scoping review was carried out from July 2015 - March 2016 to -

i) map the epidemiology, and control and prevention programmes in Hong Kong
ii) identify the evidence and assumption made in TB control
iii) guide the topics to be included for systematic review of the epidemiology and interventions in tuberculosis

Then, a preliminary logic model is being developed to illustrate how current TB control interventions impact TB outcomes in a causal link pathway. The strengths of current research and evidence gaps will also be identified and used to build this model.

PUBLIC HEALTH SIGNIFICANCE

A logic model that enables the identification of priorities areas for developing strategies and interventions to eliminate TB in a health system will be developed. We will hopefully get politicians buy in easier since the intervention matrix can be better appreciated in this evidence-based graphical format.

COMPARISON OF TUBERCULIN SKIN TEST AND QUANTIFERON-TB GOLD IN TUBE TEST IN IMMUNOCOMPROMISED PATIENTS WITH MALIGNANT HAEMATOLOGIC DISEASES

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Background: Tuberculosis (TB) is a communicable disease caused by infection with organisms of the Mycobacterium tuberculosis complex (M. tuberculosis, M. bovis, M. africanum) and an important reason of morbidity and mortality worldwide. One third of the world population has latent tuberculosis (TB) infection (LTBI) [B1]. Patients with immunodeficiencies, such as those suffering from haematologic malignancies, have a greater risk of progressing to active TB disease once infected. It is estimated that the Relative Risk of TB disease in patients with haematologic malignancies is 2-40 times that of the general population [B2]. There are currently two methods for diagnosing latent TB infection: the Mantoux tuberculin skin test (TST) and the QuantiFERON-TB Gold in-Tube test.
The specificity of the Mantoux TST is limited by cross-reactivity of the purified protein derivative (PPD) with Bacillus Calmette–Guerin (BCG) vaccine and with most of the nontuberculous mycobacteria (NTM). Its sensitivity is also low in immunocompromised patients, in whom the risk of progression to TB is high. Despite these limitations, TST is routinely used in clinical practice to screen for latent TB infection [B3]. QuantiFERON-TB Gold In Tube test (QFT-G) is used to test for cell dependent immunity (Cell Mediated Immunity - CMI) to antigenic peptides modeled with mycobacteria proteins. [B4].

Aims: The aim of the study is to diagnose LTBI in patients with haematologic malignancies by QuantiFERON-TB Gold in-Tube test compared with Mantoux TST.
Methods: The study was performed on patients with haematologic malignancies (Chronic Lymphocytic Leukemia, Chronic Myeloid Leukemia, Myelodysplastic Syndromes, Myeloproliferative Neoplasms, Multiple Myeloma, Mantle Cell Lymphoma, Hodgkin's Lymphoma, other high grade Lymphomas, Immune Thrombocytopenic Purpura) and a control group of blood donors.
All patients, attending the Hematology Unit of the Department of Internal Medicine, were enrolled consecutively. The research protocol was approved by the local ethics committee. All participants and the control group were screened for LTBI and TB disease at baseline.
A detailed medical history, duration and type of medication and data from the patients records were used. A Mantoux TST was performed along with concurrent blood sampling using the QuantiFERON method and processing it by ELISA. The QFT-GIT assay is an ELISA-based, whole-blood test that uses peptides from three TB antigens (ESAT-6, CFP-10, and TB7.7) in an in-tube format. The result is reported as quantification of IFN-gamma in international units (IU) per mL. An individual is considered positive for M. tuberculosis infection if the IFN-gamma response to TB antigens is above the test cut-off point (after subtracting the background IFN-gamma response in the negative control). We used SPSSv19.0 (SPSS Inc; Chicago IL, USA) for the statistical analysis of the results.

Results: A total of one hundred forty seven patients, eighty two men (55.8%) and sixty five women (44.2%) were tested. Median age was seventy years old (±13.114). One hundred and twenty patients had been previously treated (69.4%) with corticosteroids (prednisolone, methylprednisolone and dexamethasone) and forty five (30.6%) were treatment naive. Intense chemotherapy accompanied by long lasting agranulocytosis was given to thirty six patients (24.5%), mild to forty four patients (29.9%) and no chemotherapy to sixty seven patients (45.6%). Chemotherapy regimens included cyclophosphamide, melphalan, chlorambucil, pentostatin. Other therapies included lenalidomide, thalidomide, interferon-a, imatinib, azacitidine and other agents. Rituximab was given to nineteen patients (12.9%). Six patients (4.1%) had a positive TST (defined as ≥ 5mm indurations cut-off). There were twenty (13.6%) positive QFT-G results – one hundred twenty five (85.0%) negative and two (1.4%) indeterminate. We found a statistically significant correlation of the positivity of QTF-G with compatible with TB chest X-ray findings (2-sided $p<0.001$). Another significant correlation was that all patients with positive Mantoux TST were men (2-sided $p= 0.026$). Patients who had received corticosteroids were less frequently positive for QTF-G, compared to patients who did not receive corticosteroid (2-sided $p=0.060$). In lymphoproliferative disorders there was a statistically significant correlation of the positivity of QTF-G with compatible with TB chest X-ray (2-sided $p<0.011$) and a statistically significant correlation of the positivity of QTF-G with a compatible with TB chest X-ray (2-sided $p<0.001$) .

Summary/Conclusion: This pilot study is the first to our knowledge to compare QFT-G and Mantoux TST in immunocompromised patients with haematologic diseases and demonstrates a statistically significant correlation of the positivity of QTF-G with a compatible with TB chest X-ray. The QFT-G was a more accurate method for those infected with Mycobacterium Tuberculosis.

Keywords: QuantiFERON (QTF-G), Mantoux TS, LTBI, immunosuppression

References:

[B1] Hala A. Mohammad, Mona A. Esmail, Mohammad T. Abdelflah, Wael Soliman, Abdelfatah mohammad, Emad esmaid. Comparison of the 2-step tuberculin skin test and QuantiFERON-TB Gold in-Tube test in the screening of latent tuberculosis infection in cancer patients. Egyptian Journal of Chest Diseases and Tuberculosis 2015. 64, 681-688.

[B2] Luis Anibarro and Alberto Pena. Tuberculosis in Patients with Haematological Malignancies.Mediterranean Journal of Hematology and Infectious Diseases 2014. ISSN 2035-3006

[B3] C. Dye, c.j. Watt, D.M. Bleed, S.M. Hosscini, M.C. Roviglione. Evolution of tuberculosis control and prospects for reducing tuberculosis incidence, prevalence and deaths globally. J. Am. Med. Assoc. 2005. 293 (22) 2767-2775

[B4] Andersen, P., et al. Specific immune-based diagnosis of tuberculosis. Lancet 2000. 356; 1099-104.

INHALABLE STARCH/CARRAGEENAN MICROPARTICLES: MACROPHAGE TARGETING IN TUBERCULOSIS THERAPY

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Introduction: Tuberculosis remains a leading cause of death, therapeutic failure being mainly due to non-compliance with prolonged treatments, often associated with severe side-effects. New therapeutic strategies are demanded and, considering that the lung is the primary site of infection, direct lung delivery of antibiotics is an interesting and, possibly, effective approach. In this context, therapeutic success depends on suitable carriers that reach the alveoli where Mycobacterium hosts (macrophages)

reside, as well as on their ability to promote macrophage capture and intracellular accumulation of drugs. In this work, we propose an alternative inhalable tuberculosis therapy based on spray-dried starch/carrageenan microparticles, benefiting from the macrophage targeting ability provided by the small carrageenan (CRG) content and the safety profile of starch. The galactose content of CRG also mediates specific recognition by macrophage lectin-like receptors. Microparticles were tailored to exhibit suitable aerodynamic properties to reach the alveoli, along with geometric size of 1-2 mm, which maximizes macrophage uptake.

Results: Starch/CRG (80/20, w/w) microparticles were successfully produced by spray-drying with satisfactory production yield (above 50%). Adequate characteristics for alveolar deposition (aerodynamic diameter of 2 μm) were observed, along with spherical morphology. Microparticles successfully encapsulated an association of two first-line antitubercular drugs (isoniazid and rifabutin). Microparticles did not induce a cytotoxic effect on macrophage-like THP-1 cells. These cells demonstrated ability to uptake fluorescently-labelled CRG microparticles in a dose-dependent manner (a dose of 50 μg/cm2 elicits 82% of fluorescent cells, while 200 μg/cm2 lead to 99.9%) after an exposure of 2 h.

Conclusion: The aerodynamic properties of starch/CRG microparticles evidence their theoretical ability to reach the alveolar zone. Importantly, the observed uptake of CRG microparticles demonstrated the capacity of the material for macrophage targeting, possibly mediated by the galactose content. Overall, the results indicate the adequacy of the proposed system for inhalable antitubercular drug delivery.

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ENGINEERING A NOVEL DIAGNOSTIC TEST FOR TUBERCULOSIS USING NANOPARTICLE-BASED DETECTION OF A WHOLE BLOOD GENE EXPRESSION SIGNATURE

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Tuberculosis (TB) diagnosis remains a major public health challenge. A recent study identified a whole blood gene expression signature comprising relatively few transcripts that was able to distinguish TB from other conditions with similar presentation[1]. The same study described the disease risk score, where signals of the signature transcripts are combined into a single score suitable for test development. However, the feasibility of measuring gene expression at the point-of-care (POC) has been questioned due to technical and cost constraints.

Here, nanomaterials were used to design a POC test based on TB-specific transcript detection. The field of nanodiagnostics, in which nanoscale phenomena are linked to the presence of specific bio-analytes, has given rise to sensors with extremely low limits of detection[2]. Nanomaterials offer many signalling mechanisms, strong signal intensities, finely tuneable surface chemistries, extremely large surface areas and multiplexing capabilities.

Our detection system relies on a strand displacement of a quencher-labelled DNA probe by target mRNA, which then modulates quantum dot (QD) fluorescence emission. QDs exhibit extraordinarily bright fluorescence emission and are ideal for multiplexing due to broad excitation and narrow emission spectra[2].

The sensor design exists in two formats: a 'direct detection' format that allows sub-picomole (nanomolar) limits of detection on a standard laboratory plate reader, and an isothermal amplification-assisted format that can potentially give much lower limits of detection. Synthetic RNA targets were designed to test these sensor designs. Multiplexed detection of one up- and one downregulated signature transcript was successful in pure buffer and in the presence of purified RNA from whole blood.

This QD-based signalling mechanism is a promising advance in multiplexed mRNA detection and holds potential as a novel TB diagnostic test.

References
1. Kaforou, M., et al. (2013) PLoS Med, 10, e1001538.
2. Howes, P. D., Chandrawati, R., Stevens, M. M. (2014) Science, 346, 1247390–1247390.

HUMAN ANTIMICROBIAL RIBONUCLEASES ERADICATE MYCOBACTERIA IN A MACROPHAGE INFECTED MODEL

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Tuberculosis (TB) is an elderly infectious disease that is currently reaching one of the highest and deadliest worldwide indexes. Furthermore, this situation has been particularly aggravated by HIV coinfection, the emergence of a growing number of new cases of multi-drug resistant TB and ineffective chemotherapy treatments. Mycobacterium tuberculosis is an intracellular pathogen able to survive indefinitely under stressful conditions inside the host immune response cells. There by, the search for new anti-TB agents able to penetrate the host immune cells and eradicate the pathogen without causing damage to the host is now a priority. A mycobacterial infection denouement largely depends on the readiness of the host immune system. During infection a large assortment of antimicrobial proteins (AMPs) is released by the host immune cells to the bloodstream and nearby tissues to infected areas to counteract bacterial invasion. Because AMPs exert a potent bactericidal effect against a wide range of human pathogens and the likelihood of microbial resistance is very low, they are emerging as a new generation of natural antibiotics.

Our research group is currently working on the anti-pathogenic mechanism of action of human RNases involved in host defence. The RNase A superfamily encompasses eight functional members in humans, known as the "canonical RNases". Specifically, RNase 3, RNase 6 and RNase 7 are small highly cationic proteins secreted by eosinophils, macrophages and keratinocytes upon infection, with a demonstrated antimicrobial activity against a variety of microbes. Interestingly, Torrent et al. carried out a preliminary characterization of the RNases derived peptides, revealing that the antimicrobial mechanism of action is mostly retained at the N-terminal region of the protein [1]. Encouraged by the recent in vitro activity against mycobacteria of RNases 3 and 7 along with their N-terminal derived peptides [2], we have now adapted the developed integrated surrogate model for screening drugs against M. tuberculosis [3] in order to check the effectiveness of antimicrobial RNases at the intracellular level. The non-pathogenic and fast growing M. aurum and RAW 264.7 mouse macrophages have been used as surrogates for M. tuberculosis and human primary immune cells respectively. Results have revealed that the RNases are capable of internalizing inside the macrophages and exhibit a high antimicrobial efficacy without causing any toxicity to the host cell, corroborating their potentiality to develop alternative anti-TB therapies. Antimicrobial activities of wild type proteins have been additionally compared with their active site mutants (H15A) as well as their respective RN(1-45) peptides.

1. Torrent, M. et al. Ribonucleases as a host-defence family: evidence of evolutionarily conserved antimicrobial activity at the N-terminus. Biochem. J. 456, 99–108 (2013).
2. Pulido, D., Torrent, M., Andreu, D., Nogués, M. V. & Boix, E. Two human host defense ribonucleases against mycobacteria, the eosinophil cationic protein (RNase 3) and RNase 7. Antimicrob. Agents Chemother. 57, 3797–805 (2013).
3. Gupta, A. & Bhakta, S. An integrated surrogate model for screening of drugs against mycobacterium tuberculosis. J. Antimicrob. Chemother. 67, 1380–1391 (2012).

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