39th

Annual Congress
of the European Society
of Mycobacteriology

ESM

July 01 – 04, 2018
Dresden, Germany

Scientific Program including Abstracts

Impressum

Publisher
Agency KONSENS Ltd.
Münsterstraße 47
59368 Werne
Germany
Phone: +49 23 89 / 52 75 0
Homepage: www.agentur-konsens.de

Editor
MD PhD Daniela Maria Cirillo
Emerging Bacterial Pathogens Unit
San Raffaele Scientific Institute
via Olgettina 60
20132 Milano, Italy

Design & Layout
Agency KONSENS Ltd.

CONTENT

WELCOME MESSAGE

Dear Friends and Colleagues,

It is our great pleasure and honour to invite you to the 39th annual congress of the European Society of Mycobacteriology that will be held in Dresden, Germany, from July 01 – 04, 2018.
Tuberculosis (TB) and drug resistant TB rates are still extraordinary and TB represents a global health threat. This has been further emphasized by the G20 heads of states Communique that included TB as health priority as well as the recent WHO statement on priority pathogens. Indeed, drug-resistant TB is responsible for one-third of the world's antimicrobial resistance related deaths pointing to the importance of the fight against DR, multidrug resistant (MDR) and extremely drug resistant (XDR) TB.
This underlines the need to strengthen TB capacity building, basic and translational research in a collaborative effort. To foster this, the ESM congress 2018 aims to promote contacts between colleagues, to enable networking, exchange of knowledge and experiences, to update information in relevant and current topics, and to present new, unreckoned, fundamental, but also preliminary data.
The scientific program of the 39th ESM meeting will cover various topics of mycobacteriology: from basic science and up-to-date diagnostic methods to clinical aspects, treatment and transmission of mycobacterial infections.
Dresden is located in the valley of the River Elbe and has a long history as the capital and royal residence for the Electors and Kings of Saxony. The city is known as "Jewel Box" due to its marvellous baroque and rococo city centre, also providing cultural highlights as the Semper Oper or more than 12 museums. Dresden also developed as a cultural, educational and political centre of Germany and Europe and the Dresden University of Technology is one of the 10 largest universities in Germany and part of the German Universities Excellence Initiative.
We are not only looking forward to a stimulating congress but also to friendly and casual get-togethers in a beautiful environment.
Looking forward to see you all in Dresden, Germany.

The Scientific Committee

Daniela M. Cirillo
Sabine Hofmann-Thiel
Katharina Kranzer
Stefan Niemann
Albert Nienhaus

CONGRESS ORGANIZATION

LOCAL SCIENTIFIC COMMITTEE

Prof. Dr. Stefan Niemann
Head Molecular Mycobacteriology Group
Nat. Reference Center for Mycobacteria
Deputy Head Priority Area Infections
Forschungszentrum Borstel
Parkallee 1
23845 Borstel, Germany
E-Mail: sniemann@fz-borstel.de

Daniela Maria Cirillo, MD PhD
Head of Emerging Bacterial Pathogens Unit
San Raffaele Scientific institute
Via Olgettina 60
20132 Milano, Italy
E-Mail: cirillo.daniela@hsr.it

STEERING COMMITTEE

President

Daniela Maria Cirillo, MD PhD (Milano, Italy)

Vice-president

Troels Lillebæk, DMSc. (Copenhagen, Denmark)

Treasurer

Prof. Dr. Stefan Niemann (Borstel, Germany)

Members

Suzana David (Lisbon, Portugal)

Nalin Rastogi (Guadeloupe, France)

Ģirts Šķenders (Stopiņu novads, Latvia)

Local Organizing Agency

Agency KONSENS Ltd.

Germany

SPONSORS / EXHIBITION

We express our gratitude to all who supported and helped the organization of the 39th edition of ESM congress:

Gold Medal Sponsor

Cepheid Europe
www.cepheidinternational.com

Silver Medal Sponsors

Becton Dickinson Europe
www.bd.com

Hain Lifescience GmbH
www.hain-lifescience.com

Exhibitors

AID GmbH
www.aid-diagnostika.com

Applied Maths NV
www.applied-maths.com

AXON LAB AG
www.axonlab.com

BGW
www.bgw-online.de

Check Diagnostics GmbH
www.check-micro.de

GenoScreen
www.genoscreen.com

Insmed Ireland Limited
www.insmed.com

Ridom GmbH
www.ridom.de

Material

Artelt-Enclit
www.artelt.info

SCIENTIFIC PROGRAM

Sunday, 01.07.2018

12:00 **Registration**

14:00-16:00 **ESM - Workshop on Host Pathogen Interaction**
Chairmen: Susanne Homolka, Daniela Cirillo

Lecture 1: Impact of *M. tuberculosis* complex population diversity on the immune response and the clinical outcome of tuberculosis infection
Margarida Saraiva

Lecture 2: Host pathogen interaction : New insights into the origin and risks for the future
Philip Supply

Lecture 3: The *Mycobacterium marinum*-zebrafish model to study host-pathogen interactions in tuberculosis
Francisco J. Roca

Lecture 4: Macrophage infection models for *M. tuberculosis* virulence testing
Susanne Homolka

16:00-17:00 **Symposia organized by Industry**

16:00-16:20 Symposium 1: Cepheid
Tuberculosis screening in Migrants in Germany – the need for a new approach ?
Roland Diel

16:20-16:40 Symposium 2: BD
Molecular testing including RIF and INH resistance: changing the TB patient pathway Pre-validation of the BD MAX™ MDR-TB assay for the rapid detection of MTBc DNA and mutations associated with rifampin and isoniazid resistance
Stefan Zimmermann

16:40-17:00 Symposium 3: Hain Lifescience
Lecture: Is following

17:00-18:00 **Coffee break**

18:00-18:25 **Opening session**

18:25-18:30 **Travel Grant Awards**

18:30-19:15 **Gertrud Meissner Award ceremony**
Chairmen: Daniela Cirillo, Stefan Niemann
Lecture of the laureate

19:15-19:45 **ESM lecture**
Chairmen: Daniela Cirillo, Stefan Niemann
Lession learned from 20 years *M. tuberculosis* genome research
Roland Brosch

19:45 **Welcome reception**

20:00 **Get Together at the Industry exhibition area, International Congress Center**

Monday, 02.07.2018

9:00-10:30 **Innovation in Diagnostics – 1**
Chairmen: Katharina Kranzer, Troels Lillebaek

9:00-9:45 Guest lecture 1: Genome-based resistance prediction for surveillance
Andrea Cabibbe

9:45-10:00 OP-1: FluoroType MTB VER2.0 shows excellent sensitivity for the detection of *Mycobacterium tuberculosis* complex in smear negative specimens
Margaretha de Vos

10:00-10:15 OP-2: RNA-based drug susceptibility testing of *Mycobacterium tuberculosis*
An van den Bossche

10:15-10:30 OP-3: Sources of variation in Whole Genome Sequencing (WGS) analysis of *Mycobacterium tuberculosis*: international comparison of pipelines
Dick van Soolingen

10:30-11:00 **Coffee break**

11:00-13:00 **Innovation in Diagnostics – 2**
Chairmen: Dick van Soolingen, Ramona Groenheit

11:00-11:45 Guest lecture 2: High throughput Microtiter based MIC determination
Daniela Cirillo

11:45-12:00 OP-4: Number of effective drugs in the short course MDR-TB regimen according to standard of care drug susceptibility testing and Whole Genome Sequencing
Lennert Verboven

12:00-12:15 OP-5: Contribution of routine Whole Genome Sequencing of *Mycobacterium tuberculosis* strains to resistance detection: experience from a French center
Oana Dumitrescu

12:15-12:30 OP-6: Development of the external quality assessment scheme for non-tuberculous Mycobacteria drug susceptibility testing
Vladyslav Nikolayevskyy

12:30-12:45 OP-7: Antibiotic resistance evolution of *Mycobacterium tuberculosis* - A 12 year M/XDR-TB treatment history
Lindsay Tucker

13:00-14:00 **Lunch**

14:00-15:00 **Poster session**

15:00-16:30 **TB in vulnerable populations**
Chairmen: Annelies Van Rie

15:00-15:45 Guest lecture 3: European Outbreaks of MDR TB – View from the ECDC
Marieke J. van der Werf

15:45-16:00 OP-8: Longitudinal outbreak of multidrug-resistant tuberculosis in a hospital setting in Serbia
Matthias Merker

16:00-16:15 OP-9: Cross-border surveillance of MDR-TB circulation in Europe integrating the analysis of potential reservoirs of MDR-TB in Latin America
Dario Garcia de Viedma

16:15-16:30 OP-10: EUSeqMyTB proposed standards for Whole Genome Sequencing for *M. tuberculosis* typing in Europe
Elisa Tagliani

16:30-17:00 **Coffee break**

17:00-18:30 **Special lecture**
Chairmen: Stefan Niemann

17:00-17:45 Guest lecture 4: Insights into the evolution of tuberculosis from the study of ancient mummies and skeletons
Albert Zink

17:45-18:00 OP-11: Single-cell live confocal microscopy for monitoring lysosomal acidification induced by different *M. tuberculosis* complex lineages during macrophage infection
Paolo Miotto

18:00-18:15 OP-12: Microevolution and fitness modulation of *Mycobacterium tuberculosis* exposed to rifampicin
Charlotte Genestet

18:15-18:30 OP-13: Genomic determinants of sympatric speciation of the *Mycobacterium tuberculosis* complex across evolutionary timescales
Iñaki Comas

18:30-20:00 **Sight Seeing Tour Dresden**

20:00 **Traditional Evening at the Pulverturm**

Tuesday, 03.07.2018

09:00-10:45 New treatment concepts – 1
Chairmen: Kathleen Eisenach, Valdes Bollela

09:00-09:45 Guest lecture 5: Reversion of antibiotic resistance in *Mycobacterium tuberculosis*
Alain Baulard

09:45-10:00 OP-14: Clinical validation of intracellular pharmacodynamic based modelling for the prediction of Fluoroquinolone activity against TB
Samantha Donnellan

10:00-10:15 OP-15: TB-ULTRA, a novel 18-Plex microbead-based method for prediction of second-line drugs and ethambutol resistance in *Mycobacterium tuberculosis* complex
Bernice J. Klotoe

10:15-10:30 OP-16: Does prior clofazimine exposure exclude bedaquiline as a treatment option for MDR TB?
Farzana Ismail

10:30-10:45 OP-17: *In vitro* anti-inflammatory and antimycobacteriology activities of Echenops giganteus essential oils against two multiresistants isolates of *Mycobacterium tuberculosis*
Esther Del Florence Moni Ndedi

10:45-11:15 Coffee break

11:15-13:00 New treatment concepts – 2
Chairmen: Paolo Miotto, Leen Rigouts

11:15-12:00 Guest lecture 6: Diagnosis and treatment of M/XDR TB in an African high incidence TB/HIV setting
Gunar Günther

12:00-12:15 OP-18: Efficacy and safety of intravenous chemotherapy during intensive treatment phase in patients with newly diagnosed pulmonary tuberculosis
Dmytro Butov

12:15-12:30 OP-19: Pyrazinamide is converted into POA which is active against panD – we propose a model suggesting that this observation can finally explain everything about the unusual activity of pyrazinamide
Richard Anthony

12:30-12:45 OP-20: Acetate mediated activation of DevR dormancy regulon in *Mycobacterium tuberculosis* by acetyl phosphate metabolic signal
Saurabh Sharma

12:45-13:00 OP-21: Recombinant BCG expressing ESX-1 of *M. marinum* combines low virulence with cytosolic immune signaling and improved tuberculosis protection
Matthias Groeschel

13:00-14:00 Lunch

14:00-15:00	**Poster session**
15:00-15:30	**Coffee break**
15:30-16:15	**Gardner Middlebrook Award ceremony** Lecture of the laureate
16:15-17:15	**Population structure and transmission – 1** *Chairmen: Michael Cynamon*
16:15-16:30	OP-22: Whole Genome Sequencing as a tool to quantify local tuberculosis transmission in British Columbia, Canada *Jennifer Guthrie*
16:30-16:45	OP-23: The relationship between transmission time and clustering methods in *Mycobacterium tuberculosis* epidemiology *Conor Meehan*
16:45-17:00	OP-24: Transmission dynamics within a major *Mycobacterium tuberculosis* cluster in the Danish Kingdom spanning 23 years: What does Whole Genome Sequencing add? *Anders Norman*
17:00-17:15	OP-25: Utility of Whole Genome Sequencing (WGS) in practice *Rana Jajou*
17:15-18:30	**General assembly**
20:30	**Party at the roof terrace of the International Congress Center**

Wednesday, 04.07.2018

09:00-11:00 Population structure and transmission – 2
Chairmen: Matthias Merker, Christophe Sola

09:00-09:45 Guest lecture 8: Tuberculosis transmission in Healthcare workers
Albert Nienhaus

09:45-10:00 OP-26: Multidrug resistant *Mycobacterium tuberculosis* strains in Saudi Arbia: Myth, reality and worldwide impact
Sahal Al-Hajoj Al-Nakhli

10:00-10:15 OP-27: Using Whole Genome Sequencing to decode *Mycobacterium tuberculosis* Transmission: application in a low-burden country
Irving Cancino

10:15-10:30 OP-28: MTBseq: A comprehensive pipeline for whole genome sequence analysis of *Mycobacterium tuberculosis* complex isolates
Christian Utpatel

10:30-10:45 OP-29: Recurrent tuberculosis in patients infected with a major *Mycobacterium tuberculosis* outbreak strain in Denmark. New insights gained through Whole Genome Sequencing
Dorte B. Folkvardsen

10:45-11:00 OP-30: Key role of mass migration, as opposite to ordinary human exchange, in global spread of *M. tuberculosis* strains
Igor Mokrousov

11:00-11:30 Coffee break

11:30-13:15 One health: mycobacteria of veterinary interest
Chairmen: Albert Nienhaus

11:30-12:15 Guest lecture 9: TB insights from a One Health viewpoint
Stephen V. Gordon

12:15-12:30 OP-31: Ecology of *Mycobacterium avium* group
Ivo Pavlik

12:30-12:45 OP-32: Mycobacterial infections in Moravia and Silesia (Czech Republic) region during the years 2012-2016
Vít Ulmann

12:45-13:15 Guest lecture 10: Global *M. chimaera* outbreak – an update
Peter Keller

13:15-13:45 Poster Price Awards

13:45-14:00 Closing remarks

LIST OF ESM WORKSHOP LECTURES

L-1
Impact of *M. tuberculosis* complex population diversity on the immune response and the clinical outcome of tuberculosis infection
Margarida Saraiva

L-2
Host pathogen interaction: New insights into the origin and risks for the future
Philip Supply

L-3
The Mycobacterium marinum-zebrafish model to study host-pathogen interactions in tuberculosis.
Francisco J. Roca

L-4
Macrophage infection models for M. tuberculosis virulence testing
Susanne Homolka, Doreen Beyer, Stefan Niemann

LIST OF ESM LECTURES

ESM
Lessions learned from 20 years of M. tuberculosis genome research
Roland Brosch

LIST OF GUEST LECTURES

GL-1
Genome-based resistance prediction for surveillance of drug resistance in tuberculosis
Andrea Maurizio Cabibbe

GL-2
High throughput Microtiter based MIC determination
D.M. Cirillo on Behalf of CRyPTIC Consortium

GL-3
European outbreaks of MDR TB – View from the ECDC
Marieke J. van der Werf, Csaba Ködmön

GL-4
Insights into the evolution of tuberculosis from the study of ancient mummies and skeletons
Albert Zink

GL-5
Reversion of antibiotic resistance in *Mycobacterium tuberculosis*
Alain Baulard

GL-6
Diagnosis and treatment of M/XDR TB in an African high incidence TB/HIV setting
Gunar Günther

GL-8
Tuberculosis transmission in Healthcare workers
Albert Nienhaus

GL-9
TB insights from a One Health viewpoint
Stephen V. Gordon

GL-10
Global *M. chimaera* outbreak – an update
Peter Keller

LIST OF ORAL PRESENTATIONS (OP)

Innovation in diagnostics

OP-1
FluoroType MTB VER2.0 shows excellent sensitivity for the detection of *Mycobacterium tuberculosis* complex in smear negative specimens
Margaretha de Vos, Brigitta Derendinger, Tania Dolby, John Simpson, Grant Theron, Rob Warren

OP-2
RNA-based drug susceptibility testing of *Mycobacterium tuberculosis*
An Van den Bossche, Roby Bhattacharyya, Jean-Yves Coppee, Leen Rigouts, Alain Baulard, Deborah Hung, Vanessa Mathys, Pieter-Jan Ceyssens

OP-3
Sources of variation in Whole Genome Sequencing (WGS) analysis of *Mycobacterium tuberculosis*: international comparison of pipelines
Dick van Soolingen, Rana Jajou, Thomas Kohl, Timothy Walker, Stefan Niemann, Han de Neeling, Richard Anthony

OP-4
Number of effective drugs in the short course MDR-TB regimen according to standard of care drug susceptibility testing and whole genome sequencing
Lennert Verboven, Anzaan Dippenaar, Lesley Scott, Elise De Vos, Tim Heupink, Lynsey Isherwood, Rob Warren, Annelies Van Rie

OP-5
Contribution of routine whole genome sequencing of *Mycobacterium tuberculosis* strains to resistance detection: experience from a French center
Charlotte Genestet, Jean-Luc Berland, Christophe Ginevra, Isabelle Fredenucci, Elisabeth Hodille, Jean-Philippe Rasigade, Florence Ader, Gérard Lina, Oana Dumitrescu

OP-6
Development of the External Quality Assessment scheme for non-tuberculous Mycobacteria drug susceptibility testing
Vladyslav Nikolayevskyy, Doris Hillemann, Lucy Taylor, Helen Liddy, Yen Holicka, Katharina Kranzer

OP-7
Antibiotic resistance evolution of *Mycobacterium tuberculosis* – A 12 year M/XDR-TB treatment history
Lindsay Tucker, Matthias Merker, Doreen Beyer, Vanessa Mohr, Stefan Niemann

TB in vulnerable populations

OP-8
Longitudinal outbreak of multidrug-resistant tuberculosis in a hospital setting in Serbia
Irena Zivanovic, Matthias Merker, Elvira Richter, Thomas Kohl, Branislava Savic, Ivan Soldatovic, Thierry Wirth, Dragana Vukovic, Stefan Niemann

OP-9
Cross-border surveillance of MDR-TB circulation in Europe integrating the analysis of potential reservoirs of MDR-TB in Latin America
Fermín Acosta, Juan Agapito, Andrea Maurizio Cabibbe, Estefanía Abascal, Tatiana Cáceres, Laura Pérez-Lago, Marta Herranz, Ericka Meza, Bernice Klotoe, Patricia Muñoz, Gian María Rossolini, Alessandro Bartoloni, Pedro Valencia, Christophe Sola, Enrico Tortoli, Daniela María Cirillo, Eduardo Gotuzzo, Darío García de Viedma

OP-10
EUSeqMyTB proposed standards for whole genome sequencing for *M. tuberculosis* typing in Europe
Elisa Tagliani, Richard Anthony, Thomas A. Kohl, Vlad Nikolayevskyy, Dick van Soolingen, Stefan Niemann, Csaba Ködmön, Marieke J. van der Werf, Daniela Maria Cirillo

OP-11
Single-cell live confocal microscopy for monitoring lysosomal acidification induced by different *M. tuberculosis* complex lineages during macrophage infection
Matteo Chiacchiaretta, Nadia Bresciani, Alessandra Agresti, Samuel Zambrano, Chiara Tassan Din, Daniela M. Cirillo, Paolo Miotto

OP-12
Microevolution and fitness modulation of *Mycobacterium tuberculosis* exposed to rifampicin
Charlotte Genestet, Jean-Luc Berland, Claude Jean Baptiste, Christophe Ginevra, Elisabeth Hodille, Florence Ader, Gérard Lina, Sylvain Goutelle, Oana Dumitrescu

OP-13
Genomic determinants of sympatric speciation of the *Mycobacterium tuberculosis* complex across evolutionary timescales
Alvaro Chiner-Oms, Leonor Sánche-Busó, Jukka Corander, Sebastien Gagneux, Simon Harris, Douglas Young, Fernando Gonzalez-Candelas, Iñaki Comas

New treatment concepts

OP-14
Clinical Validation of Intracellular Pharmacodynamic based modelling for the prediction of Fluoroquinolone activity against TB
Samantha Donnellan, Ghaith Aljayyoussi, Alison Ardrey, Carmen Martinez-Rodriguez, Steve Ward, Giancarlo Biagini

OP-15
TB-ULTRA, a novel 18-Plex microbead-based method for prediction of second-line drugs and ethambutol resistance in *Mycobacterium tuberculosis* complex
Bernice J. Klotoe, Barbara Molina-Moya, Harrison Magdinier Gomes, Michel K. Gomgnimbou, Lorenna Oliveira Suzarte, Maria H. Féres Saad, Sajid Ali, José Dominguez, Edita Pimkina, Elena Zholdybayeva, Christophe Sola, Guislaine Refrégier

OP-16
Does prior Clofazimine exposure exclude Bedaquiline as a treatment option for MDR TB?
Nazir Ismail, Farzana Ismail, Netricia Govender, Lavania Joseph, Hendrik Koornhof, Shaheed Vally Omar

OP-17
In vitro* anti-inflammatory and antimycobacteriology activities of Echenops giganteus essential oils against two multiresistants isolates of *Mycobacterium tuberculosis
Maximilienne Nyegue, Esther Del Florence Moni Ndedi, Sara Eyangoh Nyobe, Steve Valdi Djova, Chantal Menut, Fraçois-Xavier Etoa

OP-18
Efficacy and safety of intravenous chemotherapy during intensive treatment phase in patients with newly diagnosed pulmonary tuberculosis
Dmytro Butov, Mykhailo Kuzhko, Mykola Gumeniuk, Tetiana Butova

OP-19
Pyrazinamide is converted into POA which is active against panD – we propose a model suggesting that this observation can finally explain everything about the unusual activity of pyrazinamide
Richard Anthony, Dick van Soolingen

OP-20
Acetate mediated activation of DevR dormancy regulon in *Mycobacterium tuberculosis* by acetyl phosphate metabolic signal
Saurabh Sharma, Priyanka Kumari, Atul Vashist, Chanchal Kumar, Malobi Nandi, Jaya Sivaswami Tyagi

OP-21
Recombinant BCG expressing ESX-1 of *M. marinum* combines low virulence with cytosolic immune signaling and improved tuberculosis protection
Matthias Groeschel, Fadel Sayes, Sang-Nae Cho, Laleh Majlessi, Roland Brosch

Population structure and transmission

OP-22
Whole Genome Sequencing as a Tool to Quantify Local Tuberculosis Transmission in British Columbia, Canada
Jennifer Guthrie, Clare Kong, David Roth, Mabel Rodrigues, Timothy Walker, Dona Foster, Patrick Tang, Victoria Cook, James Johnston, Jennifer Gardy

OP-23
The relationship between transmission time and clustering methods *in Mycobacterium tuberculosis* epidemiology
Conor Meehan, Pieter Moris, Thomas Kohl, Jūlija Pečerska, Suriya Akter, Matthias Merker, Christian Utpatel, Patrick Beckert, Florian Gehre, Pauline Lempens, Tanja Stadler, Michel Kaswa, Denise Kühnert, Stefan Niemann, Bouke De Jong

OP-24
Transmission dynamics within a major *Mycobacterium tuberculosis* cluster in the Danish Kingdom spanning 23 years: What does whole genome sequencing add?
Dorte B. Folkvardsen, Anders Norman, Erik Michael Rasmussen, Lars Jelsbak, Åse Bengaard Andersen, Troels Lillebaek

OP-25
Utility of whole genome sequencing (WGS) in practise
Rana Jajou, Han de Neeling, Arnout Mulder, Rina de Zwaan, Wim Van der Hoek, Richard Anthony, Dick van Soolingen

OP-26
Multidrug resistant *Mycobacterium tuberculosis* strains in Saudi Arbia: Myth, reality and worldwide impact
Hawra Al-Ghafli, Thomas Kohl, Bright Varghese, Matthias Merker, Stefan Niemann, Sahal Al-Hajoj Al-Nakhli

OP-27
Using Whole Genome Sequencing to Decode *Mycobacterium tuberculosis* Transmission: application in a low-burden country
Irving Cancino, Yuanwei Xu, Luis Villamayor-Cebolla, Manuela Torres-Puente, Caroline Colijn, Iñaki Comas, Valencia Region Tuberculosis Working Group

OP-28
MTBseq: A comprehensive pipeline for whole genome sequence analysis of *Mycobacterium tuberculosis* complex isolates
Christian Utpatel, Thomas Kohl, Viola Schleusener, Maria Rosaria De Filippo, Patrick Beckert, Daniela Maria Cirillo, Stefan Niemann

OP-29
Recurrent tuberculosis in patients infected with a major *Mycobacterium tuberculosis* outbreak strain in Denmark. New insights gained through whole genome sequencing
Dorte B. Folkvardsen, Anders Norman, Erik Michael Rasmussen, Troels Lillebaek, Lars Jelsbak, Åse Bengaard Andersen

OP-30
Key role of mass migration, as opposite to ordinary human exchange, in global spread of *M. tuberculosis* strains
Igor Mokrousov

One health: mycobacteria of veterinary interest

OP-31
Ecology of *Mycobacterium avium* group
Ivo Pavlik, Vít Ulmann, Jan Caha, Helena Modra, Ondrej Konecny, Dana Hübelova, Milan Bartos, Milan Gersl, Jan Kudelka

OP-32
Mycobacterial infections in Moravia and Silesia (Czech Republic) region during the years 2012-2016
Vít Ulmann, Jan Caha, Helena Modra, Ivo Pavlik

LIST OF POSTER PRESENTATIONS (P)

Host-pathogen interaction

P 112
Detailed analysis of the infection of the same MDR *Mycobacterium tuberculosis* strain in two different patients: alone or in a confection with a susceptible strain
Estefanía Abascal, Marta Herranz, Fermín Acosta, Juan Agapito, María Jesús Ruiz Serrano, Francisco Fernández-González, Paloma Gijón, Pilar Gómez Pintado, Enrique Acín, Tatiana Cáceres, Patricia Muñoz, Eduardo Gotuzzo, Darío García de Viedma

P 113
Intra-host Genetic Variability of *Mycobacterium tuberculosis* in a Patient with Pansusceptible tuberculosis
Marie Seraphin, Erik Michael Rasmussen, Anders Norman, Alexandra Gerace, Troels Lillebaek, Michael Lauzardo

P 115
Role of the Cholinergic System in Experimental Pulmonary Tuberculosis
Leon Islas-Weinstein, Jorge Barrios-Payán, Dulce Mata-Espinosa, Brenda Marquina-Castillo, Mario Alberto Zetter-Salmón, Rogelio Hernández-Pando, Iris Selene Paredes González, Octavio Ramos-Espinosa

P 117
Immunoinformatics approaches to investigate to molecular basis underlying varying adaptive immune responses induced by different *Mycobacterium tuberculosis* lineages
Carlos Magalhães, Iñaki Comas, Margarida Saraiva, Nuno Osório

P 135
Buruli ulcer disease by *Mycobacterium ulcerans* in Mexican pediatric patient
Virginia Lora-Téllez, María Lucía Pérez-Ricardez, María Fabiola Lara-Hernández, María Concepción Mella-Romero, Rigoberto Hernández-Castro, Roberto Arenas-Guzmán

P 150
Genome Sequencing and comparative genomics of 12 Brazilian *Mycobacterium kansasii* clinical isolates
Edson Machado, Philip Noel Suffys, Luciana Destasio de Carvalho, Vinicius Mussi, Elena Lasunskaia, Conor Meehan, Antonio Basílio de Miranda, Marcos Catanho

P 201
Rv3484, a LytR-cpsA-Psr protein encoding gene of *Mycobacterium tuberculosis*, is essential to establish infection in the mouse model of tuberculosis
Sven Malm, Silvia Maaß, Ulrich E. Schaible, Stefan Ehlers, Stefan Niemann

Innovation in diagnostics

P 6
Designing of a method based on reverse-hybridization line probe assay (LiPA) for rapid detection of prevalent Mycobacterium species from clinical specimens
Reza Kamali Kakhki, Ehsan Aryan, Mojtaba Sankian, Zahra Meshkat

P 7
Using novel DNA target for the rapid detection of *Mycobacterium tuberculosis* from clinical samples
Reza Kamali Kakhki, Alireza Neshani, Mojtaba Sankian, Kiarash Ghazvini, Amin Houshyar, Mahsa Sayyadi

P 15
TB-SeqDisK: a microfluidics platform in combination with whole genome sequencing for ultra-fast notification of *Mycobacterium tuberculosis* and its resistance pattern
Thomas Kohl, Markus Beutler, Matthias Merker, Tobias Paprotka, Kerstin Rönsch, Markus Schmitt, Wolfgang Grasse, Christoph Metzger-Boddien, Katharina Dormanns, Jan Lüddecke, Nils Paust, Judith Schlanderer, Lubov Delamotte, Jörg Schickedanz, Michael Steinwand, Stefan Niemann, Harald Hoffmann

P 30
Direct detection of *Mycobacterium tuberculosis* rifampin resistance in bio-safe stained sputum smears
Surabhi Lavania, Divya Anthwal, Manpreet Bhalla, Nagendra Tomar, Sagarika Haldar, Jaya Tyagi

P 37
Pervasive effect of contaminant DNA on MTB whole genome sequencing outcomes: a study across clinical settings and sample specimens
Galo A. Goig-Serrano, Iñaki Comas

P 41
Integrating functional genomics and phylogenetics for the discovery of new antibiotic resistance determinants in *Mycobacterium tuberculosis*
Victoria Furió, Miguel Angel Moreno, Álvaro Chiner-Oms, Manuela Torres-Puente, Luis Villamayor, Iñaki Comas

P 57
Validation of the SensititreTM microbroth dilution method for *M. tuberculosis* complex (MTBC) antimicrobial susceptibility testing (AST)
Nurhazirah Mohd Ya'akob, Shuwei Zheng, Kee Mong Ha, Bin Eng Cynthia Chee, Yee Tang Wang, Li-Hwei Sng

P 75
Evaluation of Xpert MTB/RIF Ultra performance for pulmonary tuberculosis diagnosis on respiratory smear-negative samples in a French center
Elisabeth Hodille, Audrey Maisson, Laurine Charlet, Bauduin Clyde, Charlotte Genestet, Isabelle Fredenucci, Jean-Philippe Rasigade, Gérard Lina

P 78
Sensitivity comparaison of two real-time PCR kits for the in vitro *Mycobacterium tuberculosis* complexe diagnosis
Elisabeth Hodille, Alexia Barbry, Charlotte Genestet, Isabelle Fredenucci, Jean-Philippe Rasigade, Gérard Lina, Oana Dumitrescu

P 85
Systematic evidence of the performance of the phenotypic non-commercial assays for the detection of *Mycobacterium tuberculosis* resistance to antituberculosis drugs
Vladyslav Nikolayevskyy, Jim Werngren, Kadri Klaos, Yen Holicka, Anh Tran, Irina Kontsevaya

P 99
Phenotypic and genotypic characterization of resistance to bedaquiline and delamanid in *M. tuberculosis* clinical strains
Simone Battaglia, Emanuele Borroni, Andrea Maurizio Cabibbe, Matteo Chiacchiaretta, Maria Rosaria De Filippo, Daniela Maria Cirillo, CRyPTIC Consortium

P 100
Comparison of Decomics Decontamination Kit with Kubica Method for Recovery of *Mycobacterium tuberculosis* from Sputum
Meltem Ozden, Zeynep Saribas, Alpaslan Alp, Mutlu Hayran, Yakut Akyon

P 106
Line Probe assay (LPA) to identify Non-tuberculous Mycobacteria (NTM) isolates in a Southern Brazil reference hospital
Angelica C.A. Silva, Vinicius R. Belga, Lucas Menon, Renata H.C. Pocente, Erica Chimara, Valdes Bollela

P 122
Genotyping of *Mycobacterium tuberculosis* by conventional and WGS based methods
Marjo Haanperä, Pieter Smit, Silja Mentula, Hanna Soini

P 123
Comparison of GeneXpert Mtb / RIF test results with culture and microscopy in pulmonary and extrapulmonary samples
Derya Altun, Ahmet Arslantürk, Hulya Simsek, Alper Sarıbaş, Nilay Uçarman

P 124
Identification of the Apa protein secreted by *Mycobacterium avium* subsp. paratuberculosis as a potential fecal biomarker for the immunodiagnosis of Johne's disease in cattle
Giliane Silva Souza, Ana Barbara Rodriguez, Maria Isabel Romano, David Gitirana Rocha, Wilmar Dias Silva, Elena Lasunskaia

P 126
Comparison with FluoroType MTBDR Assay and Culture for the Detection of *Mycobacterium tuberculosis* and Resistance to Rifampicin and Isoniazid, And Concordance between FluoroType MTBDR Assay and Phenotypic Drug Susceptibility Testing
Hulya Simsek, Derya Altun, Ahmet Arslanturk, Alper Sarıbaş, Nilay Uçarman

P 127
WGS as a tol for diagnosing drug- and multidrug-resistant tuberculosis in Mexico
Carlos Madrazo-Moya, Betzaida Cuevas-Cordoba, Jose Zarrabal, Iñaki Comas, Aurora Parissi-Crivelli, Ana JImenez-Ruano, Nancy Seraphine, Vanessa Gonzalez, Michael Lauzardo, Roberto Zenteno-Cuevas

P 141
Comparison between xpert MTB/RIF ultra and nested PCR for identification of *M. Tuberculosis* complex in formalin fixed paraffin embedded specimens
Anna Camaggi, Claudia Veggiani

P 147
Xpert Ultra – improving detection at what cost?
Shaheed Vally Omar, Farzana Ismail, Elliot Elawani Marubini, Lavania Joseph, Nazir Ismail

P 153
Lack of association of novel mutation aftB D397G with Ethambutol resistance in clinical isolates of *Mycobacterium tuberculosis* reveals the necessity of genotyping
Astha Giri, Shraddha Gupta, Andrea M. Cabibbe, Kamal Shrivastava, Anshika Narang, Alberto Trovato, Simone Battaglia, Naresh Sharma, Daniela Maria Cirillo, Mandira Varma-Basil

P 155
Whole-genome sequencing characterization of *Mycobacterium tuberculosis* drug-resistance profiles at a referral centre in Rome: implications for diagnosis and diseases control
Angela Cannas, Ornella Butera, Chiara De Giuli, Antonio Mazzarelli, Carolina Venditti, Gina Gualano, Fabrizio Palmieri, Enrico Girardi, Antonino Di Caro

P 167
Europpean inter-laboratory comparison of variable number tandem repeat typing and whole genome sequencing
Richard Anthony, Participants of EQA 2017, Rana Jajou, Dick van Soolingen, Vladyslav Nikolayevskyy

P 178
Genomic of MDR and XDR-TB in Kazakhstan by combination of high throughput methods
Bernice Klotoe, Emilyn Conceição, Sarah Kacimi, Sikhayeva Nurgul, Harrison Magdinier Gomes, Sarah Sengstake, Anna Schuitema, Arike Alenova, Elena Zholdybayeva, Richard Anthony, Guislaine Refrégier, Christophe Sola

P 183
Comparison between spoligotyping and Region of Difference PCR for identification of M. bovis and BCG strains in Turkey
Begum Kayar, Gulfer Yakici, Emel Yarar, Firat Karsli, Ali Uckayabasi, Manaf ElMatar, Tulin Gokmen, Togrul Nagiyev, Fatih Koksal

P 186
An in- house Duplex PCR assay used for identification of *Mycobacterium tuberculosis* as an abutment of MGIT TBc ID test
Chanchal Kumar, Kamal Shrivastava, Naresh Sharma, Jitender Yadav , Mandira Varma-Basil

P 192
Comparison of Newly Developed BD MAXtm MDR-TB panel and DNA sequencing method for molecular diagnosis and resistance of *Mycobacterium tuberculosis* complex strains
Gulfer Yakici, Begum Kayar, Emel Yarar, Firat Karsli, Ali Uckayabasi, Manaf Elmatar, Togrul Nagiyev, Fatih Koksal

P 204
Phenotypic and genotypic characterization of resistance to bedaquiline and delamanid in *M. tuberculosis* clinical strains
Simone Battaglia, Emanuele Borroni, Andrea M. Cabibbe, Matteo Chiacchiaretta, Maria Rosaria De Filippo, Faisal Masood, Sabira Tahseen, Daniela Maria Cirillo, Cryptic Consortium

P 217
pncA sequence analysis for confirmation of PZA resistance
Elvira Richter, Ulrich Eigner, Ingelore Frischmann, Anke Veldenzer, Michael Weizenegger

P 220
Detection of low-frequent resistance-mediating variants in tuberculosis bacteria using next-generation sequencing
Viola Schleusener, Ivan Barilar, Silke Feuerriegel, Stefan Niemann

P 221
Evaluation of Next Generation Sequencing library preparation kits based on enzymatic fragmentation for medium and high throughput whole genome analysis of clinical *M. tuberculosis* complex strains
Vanessa Mohr, Tanja Ubben, Carina Hahn, Tanja Struve-Sonnenschein, Anja Lüdemann, Christian Utpatel, Thomas A. Kohl, Stefan Niemann

P 223
Targeting *Mycobacterium tuberculosis* in the blood of patients with active and latent tuberculosis
Stefan V. Panaiotov, Vladimir Milanov, Maria Nikolova, Elena Nikolova, Rumen Dimitrov, Reni Kalfin

TB in vulnerable populations

P 33
DevS/DosS sensor is bifunctional and its phosphatase avtivity percludes aerobic DevR/DosR expression in *Mycobacterium tuberculosis*
Priyanka Kumari, Kohinoor Kaur, Saurabh Sharma, Snigdha Sehgal, Jaya Tyagi

P 66
Tuberculosis in a South African correctional centre: High mobility of detainees impedes TB control
Katie Baird

P 67
Migration and tuberculosis in the Czech Republic
Maria Müllerová

P 111
TB in indigenous peoples with settlement at puerto narino- Amazonas, Colombia
Francy Johanna Perez Llanos, Alejandro Vega Marín, Luz Mila Murcia, Clara Viviana Mape, Carlos A. Parra-López, Myriam Navarrete, Ricardo Sánchez, Stefan Niemann, Martha I. Murcia

P 136
Xpert MTB/RIF assay useful for pediatric patient tuberculosis disease diagnosis
Virginia Lora-Téllez, María Lucía Pérez-Ricardez, Maricruz Gutiérrez-Brito

P 156
Association of Expression of Negative Regulators of Human Innate Immune Response with Susceptibility to Tuberculosis in Turkish Population
Emel Eker, Togrul Nagiyev, Begum Kayar, Ali Üçkayabaşı, Gülfer Yakıcı, Firat Karsli, Fatih Koksal

P 195
Cutaneous non-tuberculosis mycobacterial infections: A six year Retrospective Evaluation
Gülnur Tarhan, Hulya Simsek

P 196
A retrospective evaluation of Mycobacterium abscessus prevelance in pulmonary diseases
Gülnur Tarhan, Hulya Simsek, Ismail Ceyhan

P 198
Retrospective Evaluation of 38 Patients with Tuberculous Pleurisy in Mersin, Turkey
Mahmut Ülger, Nurbanu Kurnaz, Seda Tezcan Ülger, Nuran DelialioĞlu, Gönül Aslan

New treatment concepts

P 3
Bio-guided Isolation of Anti-Mycobacterium ulcerans Principles from Allanblackia kisonghi Vermoesen (Clusiaceae)
Patrick Valere Tsouh Fokou, Regina Appiah-Opong, Dorothy Yeboah-Manu, Phyllis Addo, Abena Adoma Kissi-Twum, Lauve Rachel Tchokouaha Yamthe, Dorcas Osei-Safo, Fabrice Boyom, Alexander K. Nyarko

P 13
Immunogenicity of the *Mycobacterium tuberculosis* aldehyde dehydrogenases family proteins following stimulating MDRTB patients naive T-cell
Alireza Hadizadeh Tasbiti, Shamsi Yari, Morteza Ghazanfari, Morteza Masomi, Farid Abdolrahimi, Sharareh Khanipour, Shahin Pourazar, Seyed Davar Siadat

P 38
***Mycobacterium tuberculosis* cell wall: The potential target for the discovery of new therapies**
Chitra Rani, Inshad Ali Khan, Sujata Sharma, Tej Pal Singh

P 47
Antimycobacterial efficiency of three essential oils from plant currently used to treat tuberculosis traditionally tuberculosis in Cameroon
Esther Del Florence Moni Ndedi, Maximilienne Nyegue, Patrick Hervé Betote Diboue, Jean Paul Assam Assam, Veronique Penlap Beng, Fraçois-Xavier Etoa

P 59
Preliminary study of the in vitro activity of Tedizolidagainst *Mycobacterium tuberculosis* strains
Pilar Ruiz, Manuel Vaquero, Manuel Causse del Rio, Manuel Casal-Román

P 104
Effect of anti-tuberculous drugs on *Mycobacterium tuberculosis* intra-macrophagic behaviour
Charlotte Genestet, Florence Ader, Fanny Bernard Barret, Elisabeth Hodille, Gérard Lina, Sylvain Goutelle, Oana Dumitrescu

P 131
In vitro *Mycobacterium tuberculosis* mutants resistant to bedaquiline and clofazimine display cross-resistance
Nabila Ismail, Remco Peters, Nazir Ismail, Shaheed Vally Omar

P 139
Emergence of bedaquiline resistance after completion of bedaquiline-based drug-resistant TB treatment: a case study from South Africa
Margaretha de Vos, Serej Ley, Brigitta Derendinger, Anzaan Dippenaar, Melanie Grobbelaar, Anja Reuter, Johnny Daniels, Scott Burns, James Posey, Grant Theron, Rob Warren, Helen Cox

P 157
No evidence for cross-resistance between para-aminosalicylic acid and trimethoprim-sulfamethoxazole in *Mycobacterium tuberculosis*
Elena Martinez, Shaheed Vally Omar, Matthias Merker, Thomas Schön, Erik Sturegård, Danesh Moradigaravand, Julian Parkhill, Nazir Ismail, Vitali Sintchenko, Sharon Peacock, Stefan Niemann, Claudio Köser, Silke Feuerriegel

P 176
Isoniazid resistance in *Mycobacterium tuberculosis* is a heterogeneous phenotype comprised of overlapping MIC distribution with different underlying resistance mechanisms
Arash Ghodousi, Emanuele Borroni, Claudio U. Köser, Daniela Maria Cirillo

P 197
Investigation of in vitro Antituberculosis Activity in the Plant Extracts from St. John´s wort (Hypericum perforatum L.) and Aloe Vera
Efdal Oktay Gültekin, Mahmut Ülger, Erdal Yabalak, Nurbanu Kurnaz, Seda Tezcan Ülger, Nuran Delíalíoğlu, Ahmet Murat Gizir, Gönül Aslan

P 211
Xanthates: metabolsim by flavoprotein-containing monooxygenases and antimycobacterial activity
Violeta Valcheva, Tsveta Stoyanova, Paul R. Ortiz de Mantellano, Stanislav Yanev

Population structure and transmission of mycobacteria

P 5
Mycobacterial community structure in the soil environments determined with hsp65 targeted amplicon sequencing
Tomoaki Ichijo, Daisuke Shimizu, Nobuyasu Yamaguchi, Naoki Kagimura, Masao Nasu

P 22
Genomic comparison of pathogenic and non-pathogenic mycobacteria using *Mycobacterium bovis* Bacillus Calmette-Guérin as a model
Maria Carolina Sisco Zerpa, Marlei Gomes, Luciana Distásio de Carvalho, Carlos Eduardo Dias Campos, Paulo Cesar de Souza Caldas, Jesus Pais Ramos, Beatriz Lopez, Leila Mendonça-Lima, Jacobus De Waard, Rafael Silva Duarte, Philip Noel Suffys

P 51
Non-Tuberculous Mycobacterial healthcare-associated infections, a French 3-years retrospective study
Emmanuel Lecorche, Daniau Côme, Faïza Mougari, Hanaa Benmansour, Jérôme Robert, Anne Berger-Carbonne, Emmanuelle Cambau

P 63
Relative risk of all-cause mortality in patients with nontuberculous mycobacterial lung disease in US managed care
Theodore Marras, Christopher Vinnard, Keith Hamilton, Gina Eagle, Engels Chou, Raymond Zhang, Marko Obradovic, Roald van der Laan, Quanwu Zhang

P 64
Population-based incidence and prevalence of nontuberculous mycobacterial lung disease in a large US managed care health plan, 2008-2015
Kevin Winthrop, Gina Eagle, Roald van der Laan, Marko Obradovic, Raymond Zhang, Quanwu Zhang

P 114
Species Distribution of Nontuberculosis Mycobacteria Isolated from Pulmonary and Extrapulmonary Samples in Turkey (2017)
Hulya Simsek, Ahmet Arslantürk, Meryem Demir, Derya Altun, Alper Sarıbaş, Nilay Uçarman

P 130
Characterization of virulence and genome analysis of *Mycobacterium kansasii* strains isolated from patients ith pulmonary disease in Brazil
Philip Noel Suffys, Vinicius Mussi, Fabricio Almeida, Sanderson Calixto, Thatiana Ventura Simão, Luciana Distacio, Edson Machado, Marcos Catanho, Antonio Basilio de Miranda, Elena Lasunskaia

P 143
Functional analysis of essential mycobacterial cell division genes
Susanne Gola

P 164
Prevalence of aminoglycoside and macrolide resistance among Slovenian M. abscessus and M. intracelullare isolates
Sara Truden, Manca Zolnir-Dovc, Nadja Polanc

P 214
Use of Multilocus Seuqnce Typing of *Mycobacterium xenopi* to distinguish relapse from reinfection in a pulmonary patient
María Teresa Tórtola Fernández, Carlos Escartin, Miguel Fernández Huerta, Maria Carmen Vivas, Josep Vegue, Monica Mena

P 226
Differential drug susceptibility patterns of mycobacterium chimaera and other members of the *mycobacterium avium* complex
Florian P. Maurer, Philipp Pohle, Margit Kernbach, Daniela Sievert, Michael Hombach, Doris Hillemann, Katharina Kranzer

Population structure and transmission of MTB

P 14
The role of the IS6110 in micro- and macroevolution of *Mycobacterium tuberculosis* lineage 2
Egor Shitikov, A. Guliaev, J. Bespyatykh, Igor Mokrousov, E. Ilina, V. Govorun

P 40
Whole genome sequencing reveals transmission of tuberculosis between patient and guinea pig populations
Lesibana Malinga, Jeannette Brand, Elsabie de Kock, Peter van Heusden, Martie van der Wlt, Edward Nardell, Anton Stoltz

P 43
Rapid and robust detection of the epidemiologically important Central Asian/Russian strain of *M. tuberculosis* Beijing genotype
Igor Mokrousov, Anna Vyazovaya, Ekaterina Chernyaeva, Natalia Solovieva, Yuriy Skiba, Klavdia Levina, Violeta Valcheva, Weiwei Jiao, Lia Lima Gomes, Alena Gerasimova, Daria Starkova, Olga Narvskaya, Philip Noel Suffys, Marge Kütt, Adong Shen, Viatcheslav Zhuravlev

P 52
Population structure of *Mycobacterium tuberculosis* in the Komi Republic, Russian Federation
Anna Vyazovaya, Eugenia Proshina, Snezhana Toinova, Ion Avadenii, Alena Gerasimova, Natalia Solovieva, Olga Narvskaya, Viacheslav Zhuravlev, Igor Mokrousov

P 54
Ancient sublineages of *Mycobacterium tuberculosis* Beijing family in West Siberia, Russia
Anna Vyazovaya, Oksana Pasechnik, Alena Gerasimova, Vladimir Stasenko, Igor Mokrousov

P 61
MIRU-VNTR typing of multi-drug resistant *Mycobacterium tuberculosis* complex clinical strains
Antonella Grottola, Giulia Fregni Serpini, Claudio Rabacchi, Maria Luisa Simone, Filippo Ferrari, Paola Pietrosemoli, William Gennari, Sara Tagliazucchi, Giulia Forbicini, Nadia Nanni, Rita Magnani, Monica Pecorari

P 88
Redefining an outbreak: Whole genome sequencing sheds light on the transmission dynamics of a multi-drug resistant *Mycobacterium tuberculosis* outbreak over 23 years
Anzaan Dippenaar, Rob Warren, Margaretha de Vos, Tim Heupink, Annelies van Rie, Charlene Clarke, James Posey, Samantha Sampson, Elizabeth Streicher

P 92
Purifying selective pressure suggests functionality of vitamin B12 biosynthesis pathway in a global population of *Mycobacterium tuberculosis*
Alina Minias, Piotr Minias, Bożena Czubat, Jarosław Dziadek

P 96
Whole genome sequence based resistance prediction and molecular typing of *Mycobacterium tuberculosis* complex (MTBC) strains in BioNumerics
Margo Diricks, Katrien De Bruyne, Hannes Pouseele

P 109
Phylogenetic assignment and drug resistance analysis of *Mycobacterium tuberculosis* from Mozambique and Brazil with Whole Genome Sequencing
Valdes Bollela, Cinara S. Feliciano, Livia M.P. Anselmo, Evangelina I. Namburete, Emilyn C. Conceição, Anzaan Dipenaar, Robin M. Warren, Christophe Sola, Wilson Araujo Jr.

P 128
Genetic diversity among *Micobacterium tuberculosis* in Mexico
Armando Martínez, Carlos Arturo Vázquez Chacón, David Vázquez -Gónzalez, Gabriela Espejel, Claudia Elena Bäcker

P 152
Genome-based comparison of drug resistant *Mycobacterium tuberculosis* isolates causing pulmonary and extrapulmonary tuberculosis in Russia
Ekaterina Chernyaeva, Mikhail Rotkevich, Ksenia Krasheninnikova, Dmitriy Polev, Natalia Solovieva, Viacheslav Zhuravlev, Piotr Yablonsky, Stephen J. O'Brien

P 159
Characterization of *Mycobacterium tuberculosis* transmission dynamics by WGS in the metropolitan area of Rome
Angela Cannas, Ornella Butera, Carolina Venditti, Monica Sane-Schepisi, Antonio Mazzarelli, Gina Gualano, Fabrizio Palmieri, Antonino Di Caro, Enrico Girardi

P 163
Molecular-genetic characterization of strains of non-tuberculous mycobacteria recovered in Ukraine in 2016-2017
Aleksand Zhurilo, Anna Barbova, Nataliia Kampos-Rodrihes

P 179
Drug susceptibility and molecular characterization of *Mycobacterium tuberculosis* from Eastern Sudan
Yassir A. Shuaib, Matthias Merker, Eltahir Awad Khalil, Ulrich Schaible, Lothar H. Wieler, Mohammed Ahmed Bakheit, Saad El-Tiab Mohamed-Noor, Mohamed Abdelsalam Abdalla, Elvira Richter, Katharina Kranzer, Stefan Niemann

P 185
Genomic epidemiology of a major outbreak of tuberculosis related to time, space, and virulence
Dorte B. Folkvardsen, Anders Norman, Åse Bengaard Andersen, Erik Michael Rasmussen, Lars Jelsbak, Troels Lillebaek

P 194
Using TBminer to dig into 24-MIRU-VNTR and/or spoligotyping data of *M. tuberculosis* and the Whole-Genome Sequence era, the example of Pseudo-Beijing
Memona Yasmin, Jérôme Azé, Rubina Tabassum Siddiqui, Sabira Tahseen, Shahid Ahmad Abbasi, Naeem Khan, Rizwan Iqbal, Christophe Sola, Guislaine Refrégier

P 212
Assembling high-quality *Mycobacterium tuberculosis* complex genomes from short and long-read technologies
Miguel Blanco, Galo A. Goig, Manuela Torres, Irving Cancino, Iñaki Comas, Sebastien Gagneux

P 216
Nine-year molecular epidemiological study of tuberculosis in Hannover, Germany
Patrick Beckert, Mustafa Yilmaz, Ange Hanke-Lensing, Mareike Bliemeister, Silke Gerdes, Stefan Niemann

One health: mycobacteria of veterinary interest

P 27
Current status of bovine tuberculosis in Poland – 8 years after the recognition of the country free of the disease
Lukasz Radulski

P 28
Transmission of bovine tuberculosis among wild animals in Southern Poland
Lukasz Radulski

P 70
Evaluation of Interferon gamma test as one of the eradication program for bovine tuberculosis in Korea
Yun-Ho Jang, Tae-woon Kim, Min Kyu Jeong, Yu sin Ok, Bang-Hun Hyun, Jae Myung Kim

P 72
Nested-PCR for detection of *Mycobacterium bovis* in herd environmental samples
Tae-woon Kim, Yun-Ho Jang, Min Kyu Jeong, Yun Jeong Seo, Bang-Hun Hyun, Jae Myung Kim

P 224
Serological response from early and long cultures of *Mycobacterium bovis* isolates from Zacatecas, Mexico: Potential biomarkers for TBb diagnostic
Javier Jovani Sanchez Garza, Jua Manuel Favela Hernandez, Isaias Balderas Renteria, Blanca Patricia Lazalde Ramos, Sol Maria Quirarte Baez, Gloria Guillermina Guerrero Manriquez

ABSTRACTS OF ESM WORKSHOP LECTURES (L)

L-1

Impact of *M. tuberculosis* complex population diversity on the immune response and the clinical outcome of tuberculosis infection

Margarida Saraiva

Tuberculosis (TB)-causing bacteria belong to the *Mycobacterium tuberculosis* complex (MTBC), which includes seven phylogenetically distinct lineages associated with specific geographic regions in the world. Thus, the genetic diversity of *M. tuberculosis* is higher than once expected. A growing body of evidence show a functional relevance to this diversity, both in what regards the triggering of the immune response and important clinical features of TB. However, definite studies linking *M. tuberculosis* genomes, immune phenotypes and clinical outcomes are needed. Since covering the full diversity of *M. tuberculosis* in nature is virtually impossible, we devised an experimental approach started in the study of the interactions of clinical isolates of *M. tuberculosis* obtained in the region of Porto (north of Portugal) with immune cells of geographically matched human donors, relating the obtained findings with the clinics of the TB patient and then moving to the mouse model for mechanistic studies.

We show a highly homogeneous phylogenetic structure of *M. tuberculosis* in Porto, with nearly all cases (96.7%) belonging to Lineage 4 (L4). Within the L4 clade, the most represented sublineage was LAM (65.1%). At the functional level, we found that *M. tuberculosis* isolated from patients with more severe forms of TB are generally poorer triggers of the host immune response. Mechanistically, we relate this poor induction of cytokine production with a differential capacity of the different clinical isolates in activating the host inflammasome. Using the mouse model of infection, we found striking differences in the in vivo progression of infection by high versus low cytokine- inducing *M. tuberculosis* isolates, with low cytokine inducing *M. tuberculosis* isolates disseminating more than the high inducing ones.

Our studies are for the first time establishing clear bacterial genotype- immune phenotype-clinical links, offering host immune pathways and bacterial signatures to be targeted in the design of novel TB interventions.

L-2

Host pathogen interaction: New insights into the origin and risks for the future

Philip Supply

A prominent manifestation of most recent interactions between humans and the tuberculosis (TB) agents is the global rise and spread of multidrug resistant (MDR) TB strains, primarily resulting from the successive development and suboptimal effect and/or use of anti-TB compounds over the last decades. Ineffective detection and prevention of the emergence of drug resistance is favoring further resistance and bacterial mechanisms mitigating the fitness cost associated with drug resistance acquisition, resulting in extensively resistant and effectively transmissible strains. This talk will illustrate how such a concerning situation is currently developing in Southern Africa, where distinct groups of strains escaping from diagnostic algorithms being deployed globally - including selected whole genome sequencing -, are silently spreading and already gaining additional resistance to the most recently introduced anti-TB drug bedaquiline.

Retracing most ancient biological stages of the host-pathogen interaction can also contribute to identify driving factors of the global spread of the pathogen. Most of the available evidence points to a TB origin in Eastern Africa, as suggested e.g. by the geographic localization of evolutionarily early branching lineages of TB bacilli represented by *M. canettii*, which preceded the emergence of the common ancestor of the *Mycobacterium tuberculosis* complex (MTBC). Data will be presented on the identification of a new MTBC lineage present in the African Greater Lakes region, which is ancestral to the 7 other human-associated and the animal-associated lineages of the MTBC, and thus represents the most proximal step to the MTBC ancestor known to date. This finding adds new intriguing questions on the earliest stages of the host-pathogen co-evolution that took place along the Great Rift Valley.

L-3

The Mycobacterium marinum-zebrafish model to study host-pathogen interactions in tuberculosis.

Francisco J. Roca
Molecular Immunity Unit, Department of Medicine, University of Cambridge, MRC Laboratory of Molecular Biology. Cambridge CB2 OQH, UK

While Tumor Necrosis Factor (TNF) is host protective in mycobacterial infections, its excess is pathogenic. TNF excess triggers production of mitochondrial reactive oxygen species (ROS) that cause programmed necrosis (necroptosis) of infected macrophages through the participation of the mitochondrial protein cyclophilin D, a regulator of mitochondrial permeability transition. Here we show that mitochondrial ROS mediate necrosis through a signaling pathway that traverses the lysosome, cytosol, and endoplasmic reticulum (ER) before returning to the mitochondrion to execute cell death. We have identified the specific proteins, including $Ca2^{+}$ channels, which orchestrate this pathway, which we will present. In summary, we find that mitochondrial $Ca2^{+}$ overload is the ultimate feature in the pathway responsible for TNF-mediated programmed necrosis in infected macrophages. We identify currently used calcium channel blockers as well as drugs that specifically inhibit ER calcium release as pharmacological interventions that prevent pathogenic necrosis in tuberculosis.

L-4

Macrophage infection models for *M. tuberculosis* virulence testing

Susanne Homolka, Doreen Beyer, Stefan Niemann

Although members of the *Mycobacterium tuberculosis* complex (MTBC) are genetically monomorph with a genome sequence identity of higher than 99.95%, inter- and intra-lineage specific pathobiological differences play an essential role in the outcome of tuberculosis (TB) infection. The human adapted MTBC strains consist of seven distinct lineages, whereas the geographical distribution of strains of particular lineages differs markedly. *M. tuberculosis* strains belonging to e.g. lineage 4 exhibit a global distribution (generalist), while *M. africanum* strains (lineage 5 and 6) are strongly geographical restricted to West Africa (specialist). This strong phylogeographic population structure indicates that strains of particular MTBC lineages have adapted to a specific host population which shapes the outcome of infection and transmission rates. To understand the lineage specific virulence defining factors, the microenvironment provided by the host cell is an important factor. However, classical *in-vitro* macrophage infection models like the THP1 cell line system infected with the common reference strains H37Rv mimic TB infection only from a restricted point of view with no linkage to the specific host-pathogen interaction. In our previous studies, we investigated the growth behavior and the transcriptional profile of strains representing different phylogenetic lineages in blood derived human macrophages. *M. africanum* strains grew less in comparison to strains belonging to lineage 4. However, whether these growth and virulence differences are only driven by the genetic diversity of the strains investigated remains to be discussed. Therefore, we established a comparable human and bovine macrophage culture system to investigate pathogen specific factors of MTBC strains which are activated in response to the different host environment.

ABSTRACTS OF ESM LECTURES

ESM

Lessions learned from 20 years of *M. tuberculosis* genome research

Roland Brosch
Institut Pasteur, Paris, France

With the first complete genome sequence of the tuberculosis (TB) agent *Mycobacterium tuberculosis* (Mtb) published in 1998, a new era of TB research has started. It is difficult to imagine nowadays, not having immediate access to the genome-encoded information of a given gene or protein of interest. With the availability of the first mycobacterial genome information, and the development of bioinformatic tools, such as the TubercuList server, which allowed a convenient and easy access to the Mtb genome information, scientists from all over the world gained access to this precious information. The Mtb genome sequence suddenly gave novel insights into previously unrecognized new gene and protein families, metabolic pathways, hints for better understanding of the complex lipid biosynthesis and degradation pathways of Mtb. Most remarquable examples are the socalled PE and PPE, the Mce, or the Esx protein families. While by sequence similarity searches many of the open reading frames could be attributed with a putative function, the number of ORFs for which no function could be predicted remained still very high. It is clear now that over the last 20 years, work by the whole scientific community has strongly contributed to uncover many functions of these genes with previously unknown function.
Importantly, the Mtb genome sequence was also the starting point for a series of post-genomic approaches which delivered important information on the transcriptome, the proteome and the genes essential for in vitro or in vivo growth of Mtb and related members of the Mtb complex, with strong impact on host-pathogen interaction and identification of new drug targets. Initial comparative genomics approaches and the impressive development of next generation sequencing technologies in the 20 years further allowed a complete revision of the evolutionary scenario of Mtb and the Mtb complex, a concept which has then also been refined by identification of geographically relevant global lineages of Mtb strains and members of the Mtb complex. These investigations have also allowed certain line-specific traits to be identified, some of which are thought to have strongly influenced the evolutionary spread and “success” of certain Mtb lineages and/or strain families. The availability of large scale genome datasets of selected representatives of global Mtb strains/lineages nowadays allow genome-wide association studies (GWAS) to be performed which have helped to identify novel drug resistance mutations to be identified. Taken together, it is clear that the initial Mtb genome information and the results of 20 years of post-Mtb genome research have heavily enriched our knowledge about Mtb and TB, and also represent the basis for developing novel preventive and therapeutic strategies against Mtb and TB. Indeed, many lessons have been learned in these 20 years since the tubercle bacillus has stopped being a “black box”. Several new vulnerable drug targets and immunogens as well as new diagnostic approaches have been developed and the availability of the complete genome sequences was crucial for these developments. To cope with the TB pandemic, however, continued and even stronger efforts will be necessary for better understanding the many aspects of the complex interaction of Mtb bacilli with their host in addition to an efficient translation of the recently acquired knowledge into the clinical praxis and disease prevention.

ABSTRACTS OF GUEST LECTURES (GL)

GL-1

Genome-based resistance prediction for surveillance of drug resistance in tuberculosis

Andrea Maurizio Cabibbe
Emerging Bacterial Pathogens Unit, Tuberculosis Supranational Reference Laboratory, IRCCS San Raffaele Scientific Institute, Milan, Italy

Molecular-based testing represents a valuable tool for rapid and simple surveillance of drug resistance in tuberculosis (TB). This approach enables to overcome the time and technical limitations of phenotypic drug susceptibility testing that hamper the investigation of resistance in many resource-limited settings, as well demonstrated by the incorporation of Xpert MTB/RIF (Cepheid) into drug-resistance surveys of several Asian and African countries. High-throughput sequencing-based technologies have been recently introduced in some reference laboratories and used within surveys, allowing to estimate resistance to a larger number of drugs compared to the genotypic methods currently endorsed by WHO, and at a higher level of genomic resolution. The efficacy of sequencing to estimate the extent of *M. tuberculosis* complex resistance to the major first- and second-line anti-TB drugs was investigated taking 7094 isolates from population-level surveys in seven countries (Azerbaijan, Bangladesh, Belarus, Pakistan, Philippines, South Africa, and Ukraine), by a comparison of the adjusted prevalence of resistance, measured by sequencing, with the true prevalence of resistance, measured by phenotypic testing. The overall pooled sensitivity values for sequencing were 91% (95% CI 87–94) for *rpoB* (rifampicin resistance), 86% (74–93) for *katG, inhA*, and *fabG* promoter (isoniazid), 54% (39–68) for *pncA* (pyrazinamide), 85% (77–91) and 88% (81–92) for *gyrA* and *gyrB* (ofloxacin and moxifloxacin, respectively). The results showed that the high sensitivity values of sequencing can be applied to sequencing results to estimate the true prevalence of resistance for surveillance purposes, using a relatively simple statistical adjustment.
Sequencing is expected to become the standard tool for surveillance as it can be applied with high accuracy on sputum samples (targeted approach) in countries with limited culture capacity, and on isolates (whole genome) for a wider analysis. The scale-up of such procedures in resource-limited settings requires among others the: standardization of sample preparation methods, nomenclature and data analysis; establishment of quality assurance system; development of molecular biology and bioinformatics skills at local level. The support of supranational reference laboratories and continuous mentoring will be critical, similar to what has already been done in the past for phenotypic testing, as demonstrated by this study and other ongoing surveys.

GL-2

High throughput Microtiter based MIC determination

D.M. Cirillo on Behalf of CRyPTIC Consortium

In the framework of the *Comprehensive Resistance Prediction for Tuberculosis: an International Consortium* (CRyPTIC) we have designed and validated the “UKMYC5” 96-well microtitre plate able to perform MIC determination for 14 different anti-TB drugs, including bedaquiline, Delamanid and repurposed compounds. The plate is provides in dry-format for easy to transport and storage. **Validation of the layout has been performed as a multicenter study involving 8 laboratories using reference strains and a blind panel of 19 strains with different DST profile.** MICs were measured independently by two readers in each sites using the three standard methods available at 4 time points. Data showed that he optimum incubation period for a plate is 14 days. Preliminary data show that MICs measured by UKMYC5 are reproducible, comparable with other in use sensitivity detection methods and able to separate mutants from wild type population

GL-3

European outbreaks of MDR TB – View from the ECDC

Marieke J. van der Werf, Csaba Ködmön

The European Centre for Disease Prevention and Control (ECDC) collects and analyses mycobacterial interspersed repetitive units (MIRU) variable number of tandem repeats (VNTR) data from multidrug-resistant (MDR) tuberculosis (TB) cases. This allows for the identification of cross border MIRU-VNTR clusters. However,

this method is considered insufficient to identify recent transmission events and to start a cross-border cluster investigation. Recently, whole genome sequencing (WGS) has become widely available to study TB transmission. This method has a higher discriminatory power and is used for identifying and investigating cross-border clusters.
Three cross-border outbreaks of MDR TB have been investigated in 2016-2018 in the European Union (EU) (and Switzerland) using WGS information and for two outbreaks reports were published [1, 2]. Two outbreaks involved migrants from the Horn of Africa that recently entered the EU and one outbreak was linked to a university in Romania. In the outbreak investigations, WGS data were systematically collected from cases that were considered part of a potential cluster based on MIRU-VNTR information or information on specific drug resistance mutations. The WGS data allowed for excluding cases with the same MIRU-VNTR pattern from the investigation and thus limited the required follow up. These examples demonstrate how WGS can contribute to cross-border outbreak investigations.
To make full use of the potential of WGS, information on WGS patterns would need to be available close to real time at the EU level for all TB cases to allow for timely identification of molecular clusters. Such an EU level WGS database requires establishment of analysis and reporting standards as well as standardisation of WGS methodology and genotype nomenclature to ensure data comparability between data produced by different laboratories. Also a secure digital platform for the collection, storage and dissemination WGS data combined with epidemiological and clinical information is needed.
To assess the potential of using WGS at the EU level ECDC initiated a pilot project on the use of WGS for molecular typing and characterisation of MDR TB strains in the EU. The project is led by the EUSeqMyTB Consortium. This project will generate evidence on the utility of WGS for public health. ECDC will also further coordinate activities for identification of and response to cross-border outbreaks.
References

1. *Popovici O, Monk P, Chemtob D, Chiotan D, Freidlin PJ, Groenheit R, et al. Cross border outbreak of extensively drug resistant tuberculosis linked to a university in Romania. Epidemiology and Infection. Accepted.*
2. *Walker TM, Merker M, Knoblauch AM, Helbling P, Schoch OD, van der Werf MJ, et al. A cluster of multidrug-resistant Mycobacterium tuberculosis among patients arriving in Europe from the Horn of Africa: a molecular epidemiological study. Lancet Infect Dis. 2018;18(4):431-40.*

GL-4

Insights into the evolution of tuberculosis from the study of ancient mummies and skeletons

Albert Zink
Institute for Mummy Studies, EURAC Research, Bolzano, Italy

The molecular analysis of ancient pathogen DNA represents a unique opportunity for the study of infectious diseases in skeletal and mummified human remains. Within the last years, a wide range of bacterial, protozoal and viral infections have been detected in ancient tissue samples by the characterization of specific DNA fragments. This holds particularly true for the identification of the *M. tuberculosis* complex, which seems to be more robust than other microbes due to its waxy, hydrophobic and lipid-rich cell wall. These observations provided useful information about the occurrence, but also frequency of tuberculosis in former populations. Moreover, these studies present new evolutionary models and indicate the way of transmission between human and animals.
The introduction of next generation sequencing (NGS) technologies in the study of ancient human remains has made it possible to reconstruct full ancient TB genomes and thereby new important findings regarding the evolution of tuberculosis were revealed.
This development is linked to the constant adaptation of modern molecular methodologies to the needs of ancient DNA (aDNA). Particular challenging to genetic paleomicrobiology is the high abundance of environmental DNA from all sorts of microorganisms in DNA extracts from ancient bone and soft tissue samples. In this presentation, our paleopathological and molecular findings of TB cases from various geographic sites and time periods will be presented and the challenges and opportunities of molecular TB detection in ancient human remains of will be discussed.

GL-5

Reversion of antibiotic resistance in *Mycobacterium tuberculosis*

Alain Baulard

In the past decade, we have observed a dramatic worldwide increase in the number of antibiotic-resistant bacteria, against which the efficacy of the therapeutic arsenal is gradually weakened. Tuberculosis is particularly concerned by the

emergence of multidrug-resistant strains, which forces to adapt the antibiotic cocktail and to prolong the treatment, further delaying or even precluding healing. In addition, while strains resistant to all antibiotics are still exceptional, they raise concerns of a future TB epidemic with restricted therapeutic options. There is therefore an urgent medical need for new therapeutic approaches to fight more effectively against resistance.
Treatment of tuberculosis involves a large number of pro-antibiotics including isoniazid, ethionamide, pyrazinamide, delamanid, and maybe soon pretomanid. Worryingly, while resistance to the new nitro-imidazoles delamanid and pretomanid is already documented, the future of PZA is seriously compromised by the high rate of circulating resistant strains.
Our team has recently proposed a strategy to revert resistance to ethionamide, by the use of small molecules waking up alternative bioactivation pathways of this prodrug. Here we will first describe the strategy that has permit the identification of these additional activation pathways. Then, we will describe how, by extrapolating this approach to pretomanid, we identified small molecules that revert resistance to this new family of antibiotics. Possible applications of such discovery in the clinical use of nitro-imidazoles will be discussed.

GL-6

Diagnosis and treatment of M/XDR TB in an African high incidence TB/HIV setting

Gunar Günther

Only about 20% of the estimated annual MDR TB cases worldwide are currently put on treatment. The main reason is a lack of resources for diagnostics, treatment and skilled human resources in high incidence TB settings.
Namibia has currently the 5th highest TB incidence in the world and an HIV prevalence of about 14%. The incidence of drug resistant TB are rising annually.
We will report about the Namibian challenges in the management of drug resistant TB and experiences of implementation of individualized diagnostics and treatment for M/XDR TB. This comprises the establishment of a molecular diagnostics workflow, including targeted sequencing techniques in a Namibian public hospital.

GL-8

Tuberculosis transmission in Healthcare workers

Albert Nienhaus

Health workers (HW) are at increased risk for Tuberculosis (TB). The Hamburg Fingerprint Study, for instance, revealed an increased risk for recent transmission in HW with culturally confirmed TB. Therefore, HW are screened for latent TB infection (LTBI) either periodically or after unexpected contact to infectious patients or materials. These screenings may be performed either with Tuberculin Skin Test (TST) or Interferon Gamma Release Assay (IGRA), the latter most commonly used in Germany. These TB screenings revealed areas with an increased risk for LTBI, like geriatric care and infection wards. Recently a new generation of the QuantiFERON Gold in tube, one of the two commercially available IGRAs, was launched. Rather than using three antigens of the CD 4 cell row, the QuantiFERON Gold plus uses two antigens of the CD 4 and the CD 8 cell rows, each in two separated tubes. First data on using this IGRA for TB screening in HW showed high agreement between the CD4 and CD 8 response and a small increase in test variability. As the IGRA allow for a more reliable diagnosis of LTBI than the TST especially in BCG vaccinated HW, in recent years LTBI was more often notified to the social accidence insurance responsible for HW. In this presentation an overview of TB in HW in low incidence, high income countries will be given.

GL-9

TB insights from a One Health viewpoint

Stephen V. Gordon
UCD School of Veterinary Medicine, University College Dublin, Ireland

The *Mycobacterium tuberculosis* complex (MTBC) includes a menagerie of animal-adapted pathogens. What can this radiation of strains teach us about the molecular basis of MTBC pathogen evolution and host tropism, and how can a 'One Health' approach be used for improved TB control? We have focussed our work on the hallmark human- and animal-adapted MTBC strains, *Mycobacterium tuberculosis* and *Mycobacterium bovis*, respectively, and here will present work comparing these pathogens at multiple levels. These will include studies showing that while *M. bovis* is virulent and pathogenic

in a bovine experimental infection model, *M. tuberculosis* is attenuated in the same model. Also, in-depth transcriptional and proteomic profiling of *M. bovis* and *M. tuberculosis* reference strains shows how genomic differences are translated to quantitative changes in pathogen gene expression. A macrophage infection model and transcriptomic analysis were also use to explore how these pathogen-level differences impact on the cellular response to infection. Finally, the potential benefit of a One Health approach to areas such as biomarker and vaccine development will be discussed.

GL-10

Global *M. chimaera* outbreak – an update

Peter Keller

ABSTRACTS OF ORAL PRESENTATIONS (OP)

Innovation in diagnostics

OP-1

FluoroType MTB VER2.0 shows excellent sensitivity for the detection of *Mycobacterium tuberculosis* complex in smear negative specimens

Margaretha de Vos[1], Brigitta Derendinger[1], Tania Dolby[2], John Simpson[2], Grant Theron[1], Rob Warren[1]
[1]DST/NRF Centre of Excellence for Biomedical Tuberculosis Research, Sa Mrc Centre for Tuberculosis Research; Division of Molecular Biology and Human Genetics, Faculty of Medicine and Health Sciences; Stellenbosch University, Cape Town, South Africa
[2]National Health Laboratory Services, Green Point, Cape Towm, South Africa

Background: The underdiagnoses of tuberculosis (TB) remains a major hurdle for the eradication of the disease. Of the 10.4 million estimated new cases in 2016, only 6.3 million were detected and officially notified in 2016. The WHO has therefore included early diagnosis of TB and universal drug susceptibility testing as one of its core priorities for global TB control.
Objective: To evaluate the diagnostic performance of the FluoroType MTB VER2.0 (FluoroType) assay for the detection of *M. tuberculosis* complex (MTBC) in smear positive and smear negative sputum specimens.
Methods: Sputa from 94 smear-positive and 419 smear-negative patients with presumptive TB (Xpert MTB positive, n=268) were tested with the FluoroType assay using two DNA extraction methods; the FluoroLyse kit and GXT12 instrument. Each DNA extract was analysed using the FluoroType assay. Culture in combination with the Genotype MTBDR*plus* VER2.0 assay was used as the method of comparison. For discrepant results, FluoroType MTBDR, clinical data (prior exposure to anti-TB drugs) and bacillary load (TTP and Xpert Ct values) was evaluated.
Results: Preliminary analysis of the resultsshowed that the sensitivity of FluoroType for the detection of MTBC was 100% using either the FluoroLyse kit or GXT12 DNA extraction method in smear positive sputum specimens. The sensitivity and specificity for the detection of MTBC using the FluoroLyse kit was 88% and 93%, respectively in smear negative specimens. The sensitivity and specificity for the detection of MTBC using the GXT12 instrument was 86% and 93%, respectively in smear negative specimens.
Discussion: FluoroType showed excellent sensitivity for the detection of MTBC in sputum specimens and was equivalent to Xpert MTB/RIF. The assay also provides a semi-quantification result for to measure bacterial burden. Therefore, this assay provides an important tool for rapid diagnosis and identification of MTBC with the added benefit that the extracted DNA can be used for downstream drug susceptibility testing and thereby eliminating the need for the collection of a second sputum specimen.

OP-2

RNA-based drug susceptibility testing of *Mycobacterium tuberculosis*

An Van den Bossche[1], Roby Bhattacharyya[2], Jean-Yves Coppee[3], Leen Rigouts[4], Alain Baulard[5], Deborah Hung[2], Vanessa Mathys[1], Pieter-Jan Ceyssens[1]
[1]Division of Bacterial Diseases, Sciensano, Brussels, Belgium
[2]Harvard Medical School, Boston, United States
[3]Institut Pasteur, Paris, France
[4]Institute of Tropical Medicine, Faculty of Pharmaceutical, Biomedical and Veterinary Sciences, University of Antwerp, Belgium
[5]Center for Infection and Immunity of Lille, Univ. Lille, CNRS, INSERM, Chu Lille, Institut Pasteur de Lille, France

Background: To counter the long turnaround time of standard phenotypic Drug Susceptibility Testing (DST) of *M. tuberculosis* (Mtb), multiple DNA-based methods were successfully introduced over the last years. Although these are fast and sensitive, they (a) are based on the knowledge on resistance mutations which is limited for especially 2nd-line and new drugs, (b) do not distinguish living from dead cells, (c) ignore all intrinsic resistance mechanisms like efflux pump overexpression, and (d) ignore the multifactorial influence of compensatory mutations.

Here, we introduce a next-generation diagnostic test based on quantification of antibiotic-specific RNA biomarkers. The basic principle is that a brief antibiotic exposure triggers specific transcriptional responses in susceptible, but not in resistant, microbes within minutes to a few hours. A major advantage of this method is that it avoids a long culture-dependent step, yet detects the resistance phenotype, independent of the specific cause of resistance.

Materials & Methods: First, the global transcriptional response of H37Rv and clinical Mtb strains on ten anti-TB drugs including Bedaquiline, PZA and Delamanid was determined using RNAtaq-Seq. A set of highly responsive genes was selected for each drug (or drugs with a similar mode of action), and RNA-targeting probes were designed.

In a next phase, the RNA-based DST was developed in 96 well format. In short, 200 µl of a positively flagged MGIT culture is spiked with a specific concentration of one drug, while a control sample is incubated in absence of the drug. Multiplex mRNA quantification is performed directly on crude cell lysate using a combination of the bead-based MagPix™ (Luminex) and Quantigene™ Plex (Thermo Fisher) technology. Normalized, relative genes expression levels (control vs drug) are combined to one numeric value which determines the drug susceptibility of the investigated strain.

Results: We successfully developed 8 primary sets of RNA biomarkers for ten 1st-line, 2nd-line and new drugs. The assay was optimized for parameters as cell density, incubation time and lysis method. Taking isoniazid as proof of principle, we present a biomarker set of 5 responsive genes and 3 normalizing genes, which enables to distinguish susceptible, low- and high resistant Mtb strains after an incubation step of 6 hours. Next, preliminary results of the other RNA-targeting probe sets demonstrate that the biomarkers can successfully discriminate between susceptible and resistance strains for the selected drugs.

Conclusion: We present a robust, RNA-based drug susceptibility assay without the need for RNA extraction or PCR amplification. The assay was proven to be efficient for isoniazid. With a total of 8 biomarker sets under optimization, the phenotypic drug resistance profile of up to 14 drugs can be determined for any combination of antibiotics in 96 well format.

OP-3

Sources of variation in Whole Genome Sequencing (WGS) analysis of *Mycobacterium tuberculosis*: international comparison of pipelines

Dick van Soolingen[1], Rana Jajou[1], Thomas Kohl[2], Timothy Walker[3], Stefan Niemann[2], Han de Neeling[1], Richard Anthony[1]

[1]National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands

[2]Molecular and Experimental Mycobacteriology, Research Center Borstel, Germany

[3]Nuffield Department of Medicine, University of Oxford, John Radcliffe Hospital, Oxford, United Kingdom

A total of 535 samples, representing all culture positive *M. tuberculosis* complex isolates in the Netherlands from 2016, were subjected to WGS, in addition to routine VNTR typing. Cluster investigation of patients with identical VNTR profiles in 2016 was performed by municipal health services and 41 epi-links were traced. Fastq.gz files of all 535 samples were analysed in four different WGS pipelines to facilitate international comparison: 1) SNP-based method from RIVM/Bilthoven/The Netherlands; 2) SNP-based method from Oxford University/UK; 3) SNP-based method and 4) cgMLST from Borstel/ Germany.

In all pipelines, shorter than 12 SNP distances between the 41 epi-linked cases was observed. One epi-linked pair was false negative in the Bilthoven pipeline, due to poor sequence quality resulting in low coverage. In general, the genetic distances between isolates of the epi-linked cases were smaller in the Oxford and Borstel pipelines (0 – 3 SNPs), than in the Bilthoven pipeline (1 – 11 SNPs). All pipelines clustered roughly the same cases, more isolates without identified epi-links were clustered in the Oxford (n=34) and both Borstel pipelines (n= 32 in the SNP pipeline and n=39 in the cgMLST) than the Bilthoven pipeline (n=29). Not only VNTR clustered cases were clustered by WGS. Patient characteristics of isolates clustered by only WGS revealed that in some of these pairs of cases an epi-link, missed by VNTR typing, was likely.

Several differences were observed among the pipelines with regard to the version of reference genome used, software used for mapping and SNP calling, (repetitive) regions excluded in the analysis, the minimum number of reads to support SNPs, and the minimum allele frequencies. The RIVM pipeline was adapted in the light of these results to function more in line with other international laboratories pipelines, facilitating

the comparability of results.
International standardization on all these variables is necessary, and subsequently on the SNP cut-off to be applied for WGS clustering, to allow international-laboratory comparison of WGS data and reliable investigation of cross-border transmission.

OP-4

Number of effective drugs in the short course MDR-TB regimen according to standard of care drug susceptibility testing and whole genome sequencing

Lennert Verboven[1], Anzaan Dippenaar[2], Lesley Scott[3], Elise De Vos[1], Tim Heupink[1], Lynsey Isherwood[4], Rob Warren[2], Annelies Van Rie[1]
[1]***University of Antwerp, Belgium***
[2]***South African Medical Research Council Centre for Tuberculosis Research, Dst/ Nrf Centre of Excellence for Biomedical Tuberculosis Research, Stellenbosch University, South Africa***
[3]***National Health Laboratory Service, South Africa***
[4]***Department of Molecular Medicine and Haematology, National Health Laboratory Service, Johannesburg, South Africa***

Background: Patients with drug resistant tuberculosis (TB) should receive ≥ 4 effective drugs. The new short-course regimen condsists of high-dose isoniazid, pyrazinamide, prothionamide, ethambutol, kanamycin, moxifloxacin, and clofazimine. It is unknown how many drugs are effective in individuals who empirically start this regimen. We compared knowledge on drug susceptibility obtained by standard of care (SOC) methods versus whole genome sequencing (WGS) in South African patients.
Design and methods: Pre-treatment *Mycobacterium tuberculosis* isolates of 318 patients with rifampicin resistance on Xpert MTB/RIF or Hain MTBDR*plus* were assessed by SOC (Hain MTBDR*plus*, isoniazid phenotypic drug susceptibility testing (DST) in case of genotypic isoniazid susceptibility, Hain MTBDRs*l* V1) and WGS (using the genomic variants published in 2018 by Coll et al. to call resistance and variants in Rv0678 for clofazimine). Based on SOC or WGS data, isolates were classified as pan-susceptible, rifampicin mono-resistant, MDR, (pre)-XDR, or other. In absence of information, the strain was classified as susceptible to a specific drug. We estimated the proportion of patients receiving ≥4 effective drugs under the short-course regimen, with drugs with unknown resistance profiles classified as either effective or of unknown effectiveness.
Results: The distribution of resistance profiles based on SOC and WGS data is shown in Table 1. The proportion of patients receiving ≥4 effective drugs was 66% based on WGS data. This proportion was significantly overestimated by SOC methods when classifying drugs with unknown susceptibility as effective (77% vs 66%, p=0.002), and significantly underestimated by SOC methods when only classifying drugs with proven susceptibility as effective (51% vs 66% p=0.001).
Conclusion: One third of South African patients diagnosed with rifampicin resistance on Xpert MTB/RIF empirically started on the new short-course MDR-TB regimen may receive an insufficient number of effective drugs. SOC DST methods result in an inaccurate estimation of the number of effective drugs.

Table 1: ***Drug resistance profile as determined by SOC methods and whole genome sequencing in 318 South African patients diagnosed with rifampicin resistance on Xpert MTB/RIF or Hain MTBDR*****plus**

	Pan-susceptible	Rif Mono	MDR	Pre-XDR	XDR	Other
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
SOC	0 (0%)	94 (29.6%)	123 (38.7%)	35 (11.0%)	38 (11.9%)	28 (8.8%)
WGS	30 (9.4%)	68 (21.4%)	94 (29.6%)	51 (16.0%)	49 (15.4%)	26 (8.2%)

OP-5

Contribution of routine whole genome sequencing of *Mycobacterium tuberculosis* strains to resistance detection: experience from a French center

Charlotte Genestet[1], Jean-Luc Berland[2], Christophe Ginevra[1], Isabelle Fredenucci[3], Elisabeth Hodille[3], Jean-Philippe Rasigade[3], Florence Ader[4], Gérard Lina[5], Oana Dumitrescu[3]

[1]***CIRI INSERM U1111, University of Lyon, France***

[2]***Fondation Merieux, Lyon, France***

[3]***Microbiology Department, Hospices Civils de Lyon, Hôpital de la Croix Rousse, France***

[4]***Hospices Civils de Lyon - Claude Bernard Lyon 1 University; Infectious Diseases - INSERM U1111, International Center for Research in Infectiology, France***

[5]***National Reference Center for Staphylococci, Hospices Civils de Lyon, CIRI INSERM U1111, University of Lyon, France***

Background: Tuberculosis (TB) is the number one cause of mortality caused by infectious diseases all over the world. Emergence of multi drug resistant (MDR) *Mycobacterium tuberculosis* (MTB) strains requires rapid assays to overcome the turnaround time of drug susceptibility testing (DST). We implemented in our center MTB whole genome sequencing (WGS) on routine basis to fasten detection of resistant strains. Here we propose to evaluate the contribution of WGS to MTB resistance detection.

Materials/methods: Seventy-four MTB strains isolated since November 2016 (when routine WGS was implemented at the Mycobacteria Laboratory of the University Hospital Lyon, France), were included in the study. WGS was conducted on an Ilumina platform and data were processed with PhyResSE pipeline. Besides, resistance was assessed with the line probe assay (LPA) Genotype MTBDR plus V2 (Hain®) and DST was performed with the BD BACTEC MGIT SIRE and PZA kits.

Results: The gold standard for resistance to rifampicin (RIF), isoniazid (INH), ethambutol (ETB) and pyrazinamide (PZA) was based on DST results and WGS analysis when high confidence mutations were detected in rpoB, katG, inhA, embB and pncA genes. We found 2 RIF-R strains, 8 INH-R strains (5 with high level resistance and 3 with low level resistance), 3 ETB-R strains and 5 PZA-R strains.

Performances for resistance detection by the 3 tested methods are summarized below:

Antibiotic (number of resistant strains)	DST		WGS		LPA	
	Resistant strains detected	Non-detected variants	Resistant strains detected	Non-detected variants	Resistant strains detected	Non-detected variants
RIF (2)	1/2	rpoB L533P	2/2	N.A.	1/2	rpoB L533P
INH (5) (high level)	5/5	N.A.	5/5	N.A.	3/5	katG L343STOP katG Δ1-492
INH (3) (low level)	3/3	N.A.	3/3	N.A.	2/3	katG Q88P
ETB (3)	0/3	embB Q497R embB M306I	3/3	N.A.	N.A.	N.A.
PZA (5)	5/5	N.A.	5/5	N.A.	N.A.	N.A.

Conclusions: WGS only allowed accurate detection of one MDR-MTB. There was full consistency between WGS prediction of INH-R and DST results, whilst PLA predictions were false for 3/8 of INH-R strains. WGS recovered 3 ETB-R strains not detected by DST. Altogether these data support routine use of WGS for *M. tuberculosis* drug resistance detection.

OP-6

Development of the External Quality Assessment scheme for non-tuberculous Mycobacteria drug susceptibility testing

Vladyslav Nikolayevskyy[1], Doris Hillemann[2], Lucy Taylor[1], Helen Liddy[1], Yen Holicka[1], Katharina Kranzer[2]
[1]Public Health England, London, United Kingdom
[2]National Reference Centre for Mycobacteria, Research Center Borstel, Germany

Non-tuberculosis Mycobacteria (NTM) are increasingly associated with various pulmonary and extrapulmonary infections in humans. Although identification of NTMs and drug susceptibility testing (DST) of NTMs comprises a significant part of the tuberculosis (TB) reference laboratory activities in the European Union (EU) and European Economic Area (EEA), currently no internationally recognised external quality assurance (EQA) schemes exist for NTM DST. DST methods lack standardization and evidence on how to interpret DST results for specific drugs is limited.
Recognising the need for harmonization of methodologies in EU/EEA, European Reference Laboratory Network for Tuberculosis (ERLTB-Net) in 2017 conducted a pilot study among National Reference Laboratories (NRL) aimed at understanding methods employed for identification and DST and developing an EQA scheme for NTM DST
Completed questionnaires were received from a total of 32 NRLs (97.0% response rate). Thirty NRLs routinely perform identification of NTMs with a majority (77.4%; N=24) using line probe assays as a primary means of NTM speciation. Identical panels comprising 10 well characterised rapid (*M.abscessus*) and slow (*M.avium*) isolates were sent to 22 NRLs routinely performing DST for NTMs using either microdilution assays and/or Hain NTM-DR kits. EQA reports containing minimum inhibitory concentrations (MICs) and result interpretation for five key drugs (Clarithromycin (CLA) Amikacin (AMK) Moxifloxacin (MOX); Linezolid (LIN), and Doxycycline (DOX) for *M. abscessus* only) were received from 21 NRLs (95.5% response rate).
Interlaboratory agreement rates were higher for M.abscessus isolates; all five strains were found to be resistant to DOX by all NRLs (MIC 8.0 – 16.0 ug/ml). Relatively minor variations were seen in MICs and their interpretations for MOX and AMK while for LIN MIC ranges for individual isolates varied greatly (2.0 – 32.0 ug/ml) across NRLs resulting in a lower agreement with regards to results interpretation. For slow growers AMK and MOX appeared to be the most problematic drugs both in terms of MIC determination (ranging 4.0 – 64.0 and 0.5 – 8 ug/ml in individual strains, respectively) and interpretation.
This pilot study demonstrates the need for training and standardisation. Future work includes development of scoring system and criteria for a new EQA scheme.
We thank ERLTB-Net laboratories for their participation and essential help.

OP-7

Antibiotic resistance evolution of *Mycobacterium tuberculosis* – A 12 year M/XDR-TB treatment history

Lindsay Tucker[1], Matthias Merker[1], Doreen Beyer[1], Vanessa Mohr[1], Stefan Niemann[1]
[1]National Reference Centre for Mycobacteria, Research Center Borstel, Germany

Background: An infection with multidrug and extensively drug resistant (M/XDR) *Mycobacterium tuberculosis* complex (MTBC) strains requires a complex and long-term (up to 24 months) treatment. Furthermore, this treatment regimen is quite toxic with dramatically reduced treatment success rates. Here, we investigated the molecular dynamics of patient derived MTBC isolates, the correlation between drug susceptibility testing (DST), and the appearance of the resistance associated single-nucleotide polymorphism (SNP).
Methods: Eleven isolates were collected over a 12-year period from a patient exhibiting a pre-XDR turned XDR infection. These isolates underwent phenotypic drug susceptibility testing, followed by whole genome sequencing to determine drug resistance/tolerance associated mutation, fluctuation and fixation within the bacterial population.
Results: The patient was initially infected with a pre-XDR MTBC strain intrinsically resistant to isoniazid, rifampicin, pyrazinamide, streptomycin, PAS and fluoroquinolones. Throughout 12-years of treatment, the patient further developed resistance to ethambutol, ethionamide/prothionamide, capreomycin, kanamycin, and amikacin, and linezolid. Tracing the frequency of individual mutations over time, we could observe the step-wise resistance acquisition of intra-patient subpopulations and the competition with less resistant subpopulations.
Conclusion: This evolutionary arms race between bacteria and antibiotic was characterized by the rapid fixation of high confident resistance mutations and compensatory mutations. Through the investigation of sequential patient

isolates, heterogeneous subpopulations with non-canonical resistance related mutations were detected. The site at which these sub-populations arise is unclear. It is possible that subpopulations are either developing in infection sites where the bacteria are shielded from the antibiotics or only exposed to minute concentrations. These situations could be driving resistance mutations in ineffective treatment regimens. In order to further investigate this hypothesis, laboratory experiments should be conducted to show the evolution of resistance mutations emerging during minute antibiotic exposure which correlate with what is observed in the clinic.

TB in vulnerable populations

OP-8

Longitudinal outbreak of multidrug-resistant tuberculosis in a hospital setting in Serbia

Irena Zivanovic[1], Matthias Merker[2], Elvira Richter[3], Thomas Kohl[2], Branislava Savic[1], Ivan Soldatovic[4], Thierry Wirth[5], Dragana Vukovic[1], Stefan Niemann[2]
[1]Institute of Microbiology and Immunology, Faculty of Medicine, University of Belgrade, Serbia
[2]Division of Molecular and Experimental Mycobacteriology, Research Center Borstel, Germany
[3]Labor Limbach, Heidelberg, Germany
[4]Institute for Medical Statistics and Informatics, School of Medicine, University of Belgrade, Serbia
[5]EPHE / Natural History Museum, Paris, France

Although tuberculosis (TB) incidence rates decreased in Serbia, multidrug-resistant (MDR) *Mycobacterium tuberculosis* complex (MTBC) strains have been continuously isolated from TB-patients in psychiatric hospitals, and have been recently suggested to be involved in transmission routes in the country's capital Belgrade.
We employed whole genome sequencing and Bayesian statistics on all (n=103) MDR-MTBC isolates (2008-2014) in Serbia for a molecular drug resistance prediction, cluster definition and temporal calibration of phylogenetic trees. Epidemiological investigations were carried out to confirm and trace molecular clusters (i.e. country-wide transmission networks).
Overall 51.5% (51/103) of the patients were assigned to 12 molecular clusters comprising 2-14 cases. Patients diagnosed with schizophrenia were more likely to be involved in a transmission network (P = 0.0019) and 34% (35/103) of all MDR-MTBC strains were part of one TUR-genotype (lineage 4.2.2) outbreak that most likely emerged in the 1990s in one psychiatric hospital in Bela Crkva, Eastern Serbia. Furthermore analysis of the bacterial effective population size over time revealed a step wise decline of circulating MDR-MTBC strains in the country even during Yugoslav Wars (1991-1995) but also in 2005 after implementation of the Global Fund grant.
Our data show that vulnerable populations are at risk and facilitate MTBC transmission and resistance development also in countries with improved TB health care and overall decreasing TB-incidence rates. We propose that high quality TB-surveillance and diagnostics remain crucial to trace the emergence of local outbreaks.

OP-9

Cross-border surveillance of MDR-TB circulation in Europe integrating the analysis of potential reservoirs of MDR-TB in Latin America

Fermín Acosta[1], Juan Agapito[2], Andrea Maurizio Cabibbe[3], Estefanía Abascal[1], Tatiana Cáceres[2], Laura Pérez-Lago[1], Marta Herranz[4], Ericka Meza[2], Bernice Klotoe[5], Patricia Muñoz[4], Gian María Rossolini[6], Alessandro Bartoloni[7], Pedro Valencia[8], Christophe Sola[5], Enrico Tortoli[3], Daniela María Cirillo[3], Eduardo Gotuzzo[2], Darío García de Viedma[4]
[1]Instituto de Investigación Sanitaria Gregorio Marañón; Hospital General Universitario Gregorio Marañón, Servicio de Microbiología, Madrid, Spain
[2]Tb Research Unit, Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru
[3]Emerging Bacterial Pathogens Unit, Irccs San Raffaele Scientific Institute, Milan, Italy
[4]Instituto de Investigación Sanitaria Gregorio Marañón; Hospital General Universitario Gregorio Marañón, Servicio de Microbiología, Madrid; Ciberes, Ciber Enfermedades Respiratorias, Spain

[5]***Institute for Integrative Biology of the Cell (I2bc), CEA, CNRS, Univ. Paris sud, Université Paris-Saclay, France***
[6]***Clinical Microbiology and Virology Unit, Florence Careggi University Hospital, Florence, Italy***
[7]***Infectious and Tropical Diseases Unit, Careggi Hospital, Florence, Italy***
[8]***Instituto Nacional de Salud, Perú***

Objective: Multidrug-resistant tuberculosis (MDR-TB) remains a major threat. Although the highest rates of MDR-TB are concentrated in few countries, migration from these areas might export high-risk strains. Transnational efforts are required to provide a clearer picture of the cross-border transmission of MDR-TB. We selected Peru, with one of the highest rates of resistance in Latin America, and two European countries (Italy and Spain) hosting Peruvian migrants, to evaluate i) the magnitude of MDR transmission and presence of MDR-TB reservoirs in the community and inmate populations in Lima and ii) the exportation of MDR-TB from Peru to Europe via migration.
Methods: TB-SPRINT and MIRU-VNTR were applied for genotyping 60 consecutive MDR-TB cases diagnosed at the 32 health centers of San Juan de Lurigancho district (North-East Lima) in 2014-15. For comparison, we included 228 strains genotyped in the same district in 2010-11. WGS was applied for an in-depth analysis of transmission networks. Strain-specific PCRs targeting SNPs identified by WGS were developed.
Results: A high percentage of recent transmission of MDR-TB was detected in Lima (60% of the isolates in 2014/5), involving strains already present in the country four years before, which suggested the existence of a reservoir of MDR-TB. One of these strains was also identified in Peruvian migrants in Spain and Italy, being responsible for a MDR-TB outbreak in Florence, currently active, involving 11 cases spanning 11 years (0-2 SNPs pairwise distances). The study in inmates identified two MDR-cases in Madrid, transferred from a prison in Lima in 2016 and 2017, to complete their sentence in Spain, who were infected by the same strain. A third case was diagnosed in Madrid in 2018 with the same MDR strain, who stayed in the same Peruvian prison in 2005, suggesting a persistent reservoir of a MDR strain in that prison. One of these MDR cases was coinfected with a second susceptible strain, suggesting high rates of overexposure in that prison. Allele-specific PCRs were designed and transferred to Peru, Italy and Spain, to be shared to allow a simple, rapid and simultaneous cross-border surveillance of these MDR strains.
Conclusions: Thanks to an integrative transnational analysis, we detected a serious problem of active transmission of MDR-TB in Lima, which recreated similar uncontrolled transmission events after exportation of a MDR strain to Europe via migration. We also detected the importation to Europe of cases exposed to a MDR strain persistent in a Peruvian prison. Strain-specific PCRs were designed and transferred for a simplified surveillance of MDR strains involved in inter-continental outbreaks.
Funding: ERANET-LAC(ELAC2015/T08-0664,E035-ERAnet-LAC/J110-2016/FONDECYT,PER2012-ELAC2015/T08-0664),ISCIII(AC16/00057,FIS15/01554, 13/01207). Miguel Servet (CP15/00075) IFARHU-SENACYT (270-2016-293) grants for LPL/FA.

OP-10

EUSeqMyTB proposed standards for whole genome sequencing for *M. tuberculosis* typing in Europe.

Elisa Tagliani[1], Richard Anthony[2], Thomas A. Kohl[3], Vlad Nikolayevskyy[4], Dick van Soolingen[2], Stefan Niemann[3], Csaba Ködmön[5], Marieke J. van der Werf[5], Daniela Maria Cirillo[1]
[1]***Emerging Bacterial Pathogens Unit, Division of Immunology, Transplantation and Infectious Diseases, IRCCS San Raffaele Scientific Institute, Milan, Italy***
[2]***RIVM, Bilthoven, Netherlands***
[3]***Division of Molecular and Experimental Mycobacteriology, Research Center Borstel, Germany***
[4]***Public Health England, London, United Kingdom***
[5]***European Centre for Disease Prevention and Control, Stockholm, Sweden***

The rapid identification of emerging multidrug-resistant (MDR) tuberculosis (TB) clones and the accurate tracing of MDR-TB transmission chains using highly discriminatory molecular typing is critical for the development of optimal TB control and prevention strategies. Whole Genome Sequencing (WGS) based on Next Generation Sequencing technologies promises to revolutionise the diagnosis and epidemiological analysis of *Mycobacterium tuberculosis* complex (*Mtbc*) strains. The unprecedented level of discrimination achieved by WGS, which allows distinguishing between closely related strains based on single nucleotide differences, makes it a key tool for *Mtbc* genotyping.
Continuous decline of WGS costs as well as availability of bench-top and user-friendly sequencing platforms and bioinformatics tools for WGS data analysis have contributed to a wider use of WGS for diagnostic and surveillance

purposes in TB reference laboratories in the European Union (EU)/European Economic Area (EEA).
In September 2017, the European Centre for Disease Prevention and Control (ECDC) initiated a pilot study on the use of WGS for molecular typing and characterisation of *Mtbc* strains in Europe. The project, implemented by the EUSeqMyTB Consortium, aims to generate information on the feasibility and performance of WGS for MDR-TB surveillance, outbreak identification and cross-border outbreak verification. Importantly, one of the specific goals of the study is to build consensus on all pre-analytical, analytical and post-analytical phases of the WGS methodology to ensure comparability of WGS-derived data across the European TB laboratory network.
To this purpose, in November 2017 the EUSeqMyTB Consortium convened a team of technical experts to discuss and define the standards, parameters, and rules to be followed during the study. The experts addressed all the key phases of WGS, including (i) wet laboratory procedures; (ii) quality control of raw WGS data; (iii) analysis pipeline; (iv) interpretation of the sequencing data; (v) WGS and epi-data storage; (vi) reporting of WGS results; and (vii) data protection related issues.
We will present the results of the agreed standards and how these will be used in the pilot study on the use of Whole Genome Sequencing for molecular typing and characterisation of *M. tuberculosis* for the preparation of genomic surveillance of MDR-TB in the EU/EEA.

OP-11

Single-cell live confocal microscopy for monitoring lysosomal acidification induced by different *M. tuberculosis* complex lineages during macrophage infection

Matteo Chiacchiaretta[1], Nadia Bresciani[1], Alessandra Agresti[2], Samuel Zambrano[2], Chiara Tassan Din[1], Daniela M. Cirillo[1], Paolo Miotto[1]
[1]Emerging Bacterial Pathogens Unit, Division of Immunology, Transplantation and Infectious Diseases, IRCCS Ospedale San Raffaele, Milano, Italy
[2]Chromatin Dynamics Unit, San Raffaele University and Scientific Institute, Milano, Italy

One of the main features of *M. tuberculosis* (Mtb) is its ability to block the phagolysosome maturation during macrophage infection. We compared phagolysosome induction by clinical isolates with different genetic background during *in vitro* THP-1 derived macrophage-like cells infection.
We compared LysoSensor Green (ThermoFisher) signal from lysosomes of classically activated (M1) and alternatively activated (M2) THP-1 derived macrophages infected with Africanum (lineage L6), Beijing (L2), H37Rv (L4), H37Ra (L4), and CDC1551 (L4) strains during live imaging confocal microscopy (MOI 10:1) at 6 hours post-infection (p.i.). Bacteria were engineered to express the fluorescent protein dsRed. Image acquisition was carried out on a Leica TCS SP5 confocal microscope (63x, 1.40 NA immersion oil obj.; 0.89 µm x 7 stacks). Images were processed with ImageJ software to perform single-cell analysis and compare infected cells *vs* non-infected cells. Results were grouped by considering phylogenetic (ancient: L6; modern: L2, L4) and pathogenic features (more virulent: Beijing, CDC1551; less virulent: Africanum, H37Ra).
Overall, 2539 cells (1254 M1; 1285 M2) were considered for the analysis. Infection induced a statistically relevant increase of the number of lysosomes per cell compared to non-infected cells in both M1 and M2 macrophages ($P < 0.05$). Infection also induced a statistically relevant increase of the LysoSensor mean signal in both M1 and M2 macrophages ($P < 0.05$), suggesting an increased acidification of the lysosomes, but M1 macrophages displayed a more homogeneous behaviour to infection challenge compared to M2 macrophages. More virulent strains were associated with higher acidification of the lysosomes in both non-infected and infected M2 macrophages ($P < 0.05$). No differences were found comparing isolates according to phylogeny.
Our data provide evidence that more virulent strains are associated with higher acidification levels at earlier time points of infection compared to less virulent strains, suggesting that intracellular survival strategies could step in at different time points or could be even different among strains. The fact that there was also a difference between the signals from non-infected cells suggests that more virulent strains are able to induce surrounding cells. These differences were found to be statistically significant in M2 macrophages, highlighting a substantial difference in the fate of natural infection in the lung at single-cell level.

OP-12

Microevolution and fitness modulation of *Mycobacterium tuberculosis* exposed to rifampicin

Charlotte Genestet[1], Jean-Luc Berland[2], Claude Jean Baptiste[2], Christophe Ginevra[1],

Elisabeth Hodille[3], Florence Ader[4], Gérard Lina[5], Sylvain Goutelle[4], Oana Dumitrescu[3]
[1]CIRI INSERM U1111, University of Lyon, France
[2]Fondation Merieux, Lyon, France
[3]Microbiology Department, Hospices Civils de Lyon, Hôpital de la Croix Rousse, France
[4]Hospices Civils de Lyon - Claude Bernard Lyon 1 University; Infectious Diseases - INSERM U1111, International Center for Research in Infectiology, France
[5]National Reference Center for Staphylococci, Hospices Civils de Lyon, CIRI INSERM U1111, University of Lyon

Background: Tuberculosis (TB) is the number one cause of mortality caused by infectious diseases all over the world. The dosage design of current TB drug regimens recommended by the WHO has not been reviewed since the 1970s; moreover recent studies have highlighted that the current daily dose of rifampicin (RIF) in TB treatment may not be optimal. Inappropriate treatment can facilitate selection of variants with improved fitness and enhanced capacity to cause disease, and may lead to the *de novo* generation of multidrug resistant *Mycobacterium tuberculosis* (MTB).
Materials/methods: We performed SNP detection on a clinical Beijing strain after rifampicin treatment at subinhibitory doses. We therefore explored the impact of the obtained mutation on RIF susceptibility, by time-kill experiments. Fitness of mutant strains was assessed in macrophage infection model. We quantified different steps of the infection (internalization, the intra-macrophage MTB traffic and bacterial persistence) by CFU count and confocal microscopy.
Results: Upon RIF exposure, besides rpoB located SNPs (associated with antibiotic resistance), we detected SNPs leading to non-synonymous mutations in loci involved in the biosynthesis of lipids from the mycobacterial outer membrane. These mutated strains were not resistant to RIF (unchanged minimum inhibitory concentration (MIC)), however their growth was improved in presence of RIF sub-MICs. As the lipids synthetized by the loci harboring the detected SNPs are involved in the interactions of MTB with its host, we characterized the fitness of mutated strains during human macrophage infection. We observed an increase of macrophage invasion and intramacrophagic multiplication, probably due to an enhanced phagolysosomal escape and decreased autophagy activation and autolysosome formation.
Conclusions: Our experiments pointed to a significant genetic evolution of MTB treated with rifampicin (major anti-TB drug). This evolution consists in the emergence of SNP variants with potential effect on the production of MTB membrane structure lipids formerly shown as fitness determinants. The fact that anti-TB treatment may lead, under certain circumstances, to the emergence and growth of a more fitted MTB variants may explain failures of the current treatment and re-engage the question of the optimal dose of rifampicin for TB treatment.

OP-13

Genomic determinants of sympatric speciation of the *Mycobacterium tuberculosis* complex across evolutionary timescales

Alvaro Chiner-Oms[1], Leonor Sánche-Busó[2], Jukka Corander[3], Sebastien Gagneux[4], Simon Harris[2], Douglas Young[5], Fernando Gonzalez-Candelas[6], Iñaki Comas [7]
[1]Joint Research Unit "infection and Public Health" FISABIO/University of Valencia, Spain
[2]Wellcome Trust Sanger Institute, United Kingdom
[3]University of Oslo; University of Helsinki
[4]Swiss TPH; University of Basel, Switzerland
[5]The Francis Crick Institute, United Kingdom
[6]University of Valencia, I2SysBio, Evolution and Health, Spain
[7]Biomedicine Institute of Valencia, Tuberculosis Genomics Unit, Spain

Models emanating from population genomics data predict that sympatric speciation among bacteria occupying the same ecological niche is likely to leave measurable genetic signatures in extant genomes. How these models apply to professional human pathogens can have direct impact the identification of major virulence factors. Here we show that the *Mycobacterium tuberculosis* complex (MTBC) emerged through a two-stage process involving multiple core pathogenesis functions including well recognized virulence loci. In addition, a particular gene, *phoR*, played critical and changing roles in the events leading to the differentiation and later diversification of MTBC from its mycobacterial ancestor. First, during early diversification of the MTBC, *phoR* played a critical role defining the host range of the various MTBC members. Later, following adaptation to the human host, *phoR* mediates host-pathogen interaction during human-to-human transmission. Our study shows that linking pathogen evolution across evolutionary and epidemiological timescales leads to the identification of past and present virulence determinants and potential biomedical targets.

OP-14

Clinical Validation of Intracellular Pharmacodynamic based modelling for the prediction of Fluoroquinolone activity against TB

Samantha Donnellan[1], Ghaith Aljayyoussi[1], Alison Ardrey[1], Carmen Martinez-Rodriguez[2], Steve Ward[1], Giancarlo Biagini[1]
[1]Research Centre for Drugs & Diagnostics, Liverpool School of Tropical Medicine, United Kingdom
[2]Institute of Infection and Global Health, University of Liverpool, United Kingdom

Clinical studies of new anti-tubercular drugs are costly and time consuming. Owing to the extensive TB treatment periods, the ability to predict drug activity in the early development stage is vital. Recent failures of pre-clinical models in predicting the activity of fluoroquinolones in the clinic underlines the importance of developing new predictive tools that will optimise the design of future trials. Previously, we have developed an in vitro screening assay and shown that pharmacodynamic intracellular modelling (*PDi*) can be a powerful tool at predicting the activity of first line anti-TB drugs in patients. Here we show moxifloxacin (MXF) to be the superior fluoroquinolone, and *PDi* modelling based Monte-carlo simulations have accurately predicted clinical outcomes when validated against 8 independent human trials. Additionally, our simulations may explain why patients had a higher rate of relapse in the 4-month MXF trial compared to the standard regime. Based on our results, we suggest that a 5-month MXF treatment period would be superior to the standard regime with a lower relapse rate.

OP-15

TB-ULTRA, a novel 18-Plex microbead-based method for prediction of second-line drugs and ethambutol resistance in *Mycobacterium tuberculosis* complex

Bernice J. Klotoe[1], Barbara Molina-Moya[2], Harrison Magdinier Gomes[3], Michel K. Gomgnimbou[4], Lorenna Oliveira Suzarte[1], Maria H. Féres Saad[5], Sajid Ali[6], José Dominguez[2], Edita Pimkina[7], Elena Zholdybayeva[8], Christophe Sola[1], Guislaine Refrégier[1]
[1]I2bc, CEA, CNRS, Univ. Paris-sud, Université Paris-saclay, France
[2]Hospital Universitari Germans Trias Universitat Autònoma de Barcelona, Badalona, Spain
[3]I2bc, CEA, CNRS, Univ. Paris-sud, Université Paris-saclay, France; Oswaldo Cruz Foundation, Rio de Janeiro, Brazil
[4]I2bc, CEA, CNRS, Univ. Paris-sud, Université Paris-saclay, France; Centre Muraz, Bobo-Dioulasso, Burkina-Faso; Univ. Polytech, Bobo-Dioulasso, Burkina Faso
[5]Oswaldo Cruz Foundation, Rio de Janeiro, Brazil
[6]Quaid-I-Azam University, Islamabad Pakistan
[7]Infectious Diseases and Tuberculosis Hospital, Vilnius, Lithuania
[8]National Center for Biotechnology, Astana, Kazakhstan

Several diagnostic tests are being developed to detect drug resistance in tuberculosis. In line with previous developments detecting rifampicin and isoniazid resistance using microbead-based systems, we present here an assay called TB-ULTRA to detect mutations involved in second-line drugs (fluoroquinolones and three injectable drugs kanamycin, amikacin and capreomycin), and in ethambutol resistance in *Mycobacterium tuberculosis*. The proposed test includes both wild-type and mutant probes, and interpretation relies on signal comparison. Basic analysis can be performed manually. An upgraded interpretation is made available in Excel.
Using a reference set of 61 DNA extracts, we show that TB-ULTRA provides perfect concordance with pyrosequencing. Concordance between phenotype prediction and phenotypic DST was relatively good (72 to 98% concordance), with lower efficiency for fluoroquinolones and ethambutol due to some untargeted mutations. When compared to phenotypical resistance, performances were in our hands higher than those obtained with Hain MTBDRsl, possibly thanks to the use of automatized processing of data.
When applied on three uncharacterized sets, phenotype could be predicted for 51% to 98% depending on the setting and the drug investigated, detecting one extensively drug-resistant isolates in each of a Pakistanese and a Brazilian sample from which phenotypic XDR had been excluded.

The TB-ULTRA may contribute to large-scale surveillance of resistant tuberculosis and contributes to provide a clarified picture of injectable second-line drugs resistance in settings using microbead-based technologies. Interpretation method can serve as an example for other developments.

OP-16

Does prior Clofazimine exposure exclude Bedaquiline as a treatment option for MDR TB?

Nazir Ismail[1], Farzana Ismail[1], Netricia Govender[2], Lavania Joseph[2], Hendrik Koornhof[2], Shaheed Vally Omar[2]
[1]Centre for Tuberculosis, National Institute for Communicable Diseases, National Health Laboratory Services; Department of Medical Microbiology, University of Pretoria, South Africa
[2]Centre for Tuberculosis, National Institute for Communicable Diseases, National Health Laboratory Services, South Africa

Background: Bedaquiline (BDQ) is a novel anti-tuberculosis agent that has great potential to improve MDR-TB outcomes. Cross resistance to clofazimine (CFZ) has been documented and has important implications to the future utility of BDQ. This is especially true with the World Health Organization endorsement of the new short regimen which includes clofazimine as a core drug in MDR-TB therapy. We sought to evaluate prior CFZ exposure and BDQ minimum inhibitory concentration (MIC).
Materials/methods: Patients from Kwa-Zulu Natal (South Africa), initiated on BDQ therapy and underwent routine BDQ MIC surveillance testing between 2016 and 2017 were included. Consecutive patient files were retrospectively reviewed seeking patients with prior clofazimine exposure. A convenience sample size of 40 patients with prior CFZ was chosen and duration of exposure recorded. The MIC data was log2 transformed and a linear regression performed.
Results: The majority patients were male (26/40;65%) and the median age was 32 years (IQR:26-38). Prior exposure to CFZ ranged between 3 and 36 months. The results were validated for 37 of the 40 (93%) isolates. The MICs were in the wild type range for 97% (36/37) of the isolates. Regression analysis did not show any relationship between MIC and months of CFZ exposure(p=0.44).
Conclusions: Prior clofazimine exposure did not result in an increase in BDQ MICs in clinical isolates of this cohort of patients even if exposure was prolonged. Thus patients previously treated with the MDR-TB short regimen should still receive BDQ if indicated unless resistance to BDQ is detected and microbiologically confirmed. Further studies are required to assess the CFZ drug concentration achieved in Mycobacterium tuberculosis infected lung lesions.

OP 17

In vitro* anti-inflammatory and antimycobacteriology activities of Echenops giganteus essential oils against two multiresistants isolates of *Mycobacterium tuberculosis

Maximilienne Nyegue[1], Esther Del Florence Moni Ndedi[2], Sara Eyangoh Nyobe[3], Steve Valdi Djova[2], Chantal Menut[4], Fraçois-Xavier Etoa[5]
[1]Department of Microbiology, University of Yaounde 1, Cameroon
[2]University of Yaounde 1, Cameroon
[3]Centre Pasteur du Cameroun, Yaounde, Cameroon
[4]University of Montpellier, France
[5]Department of Biochemistry, Faculty of Sciences, University of Douala, Cameroon

Inflammation leading to cells necrosis is the response of lungs tissues to *Mycobaterium tuberculosis* invasion and immune reactions during tuberculosis infection. The success in tuberculosis resistant treatment showed that one of the great challenges is to find new antibiotics which target both multiresistant and extensive drug resistant strains and even more aid to repair tissues damages. Plant essential oils are mixtures of natural components with multipotential activities can acting by inhibiting bacterial invasion and metabolism disorders related to illnesses such as inflammation. The designed study aims to show the *in vitro* anti-inflammatory and antimycobacterial capacity of *Echinops giganteus* Roots essential oils. The essential oil is obtained by hydrodistillation using Clevenger apparatus and chemical composition of essential oils was determined simultaneously by Gas Chromatography (GC) and Gas Chromatography coupled to Mass Spectrometry (GC/MS). Anti-inflammatory activity of *Echinops giganteus* roots essential oil was performed by using bovine serum albumin denaturation and inhibition of anti-proteinase action methods. The anti-mycobacterial activity of these essential oils was evaluated against three isolates of *Mycobacterium tuberculosis* (one sensitive to all drugs, one multiresistant and one extensively drug resistant) using the microdilution method. The chemical analysis of the essential oils showed the presence of silphiperfol-6-ene (27,40%),

silphiperfolan-6-α-ol (11,30%), persilphiperfol-7-ène (7,40%) 7-épi-silphiperfol-5-ene (6,02%) et caryophyllene (6,96%). The essential oils exhibited anti-inflammatory activity by proteinase inhibition action and BSA denaturation with respective IC_{50} of 2.99 µg/mL and 3.43 µg/mL. *Echinops giganteus* roots essential oil exhibited good antimycobacterial activity with minimal inhibitory concentration of 52.08 µg/mL against sensitive and multiresistant isolate and 39.06 µg/mL extensively drug resistant isolate. the results showed good mycobacterial inhibition growth and potent inhibitory activity against the adverse effects of the inflammation such as tissue damage and protein denaturation during *Mycobacterium tuberculosis* infection.

OP-18

Efficacy and safety of intravenous chemotherapy during intensive treatment phase in patients with newly diagnosed pulmonary tuberculosis

Dmytro Butov[1], Mykhailo Kuzhko[2], Mykola Gumeniuk[3], Tetiana Butova[4]
[1]Kharkiv National Medical University, Ukraine
[2]National Institute on Phtysiatry & Pulmonology Named by F.G. Yanovsky NAMS of Ukraine
[3]SO "National Institute of Phthisiology and Pulmonology named after F. G. Yanovskyi NAMS of Ukraine", Ukraine
[4]Kharkiv National Medical University; Kharkiv Municipal Ambulatory №18; V. N. Karazin Kharkiv National University, Ukraine

The purpose of our study was to examine the efficacy and safety of intravenous chemotherapy during intensive treatment phase for patients with newly diagnosed pulmonary tuberculosis (pulmonary TB).
Materials and methods: The study involved 92 patients with newly diagnosed pulmonary TB aged between 20 years and 68 years. All patient with newly diagnosed pulmonary TB and chemosensitive tuberculosis were enrolled in this study. The patients were allocated to two groups. The first (control) group of 46 patients received standard chemotherapy orally. The second (main) group consisted of 46 patients who were prescribed isoniazid, rifampin, ethambutol by i / v transfusion, and pyrazinamide orally as a part of the standard treatment.
Results: Symptoms of intoxication in pulmonary TB patients from the second group were eliminated faster (1.42±0.35) of a month than the same symptoms of the group 1-(2.96±0.24) of the months,$p<0.05$; disappearance of respiratory symptoms of the group 2-(1.34±0.29) of a month, group 1-(2.65±0.43) of the months,$p<0.05$. In the group 2, the bacterioexcretion time was reducing faster and up to 2 months it reached 37(80.43±5.85%) while the time for the control group reached 25(54.35±7.34%),$p<0.05$. Destruction healing and healing frequency of destruction cavities up to 4 months amounted to 38(82.61±5.59%) (in control group - 28(60.87±7.20%),$p<0.05$) and residual changes were reducing (small changes or absence of even minimal radiological changes were found in 29(63.04 ±7.12%) patients versus 18(39.13±7.20%) of the group 1, and large residual changes accordingly in 17(36.96±7.12%) and 28(60.87±7.20%),$p<0.05$.
Conclusions: Thanks to i/v chemotherapy clinical manifestations of the in patients with pulmonary TB were eliminated faster, severe side effects to anti-TB drugs were not noticed, time of bacterial excretion and healing destruction reduced, healing frequency of destruction cavities increased and the residual changes decreased.
Keywords: tuberculosis, pulmonary tuberculosis, treatment of tuberculosis, effectiveness of treatment for tuberculosis

OP-19

Pyrazinamide is converted into POA which is active against panD – we propose a model suggesting that this observation can finally explain everything about the unusual activity of pyrazinamide

Richard Anthony[1], Dick van Soolingen[2]
[1]RIVM, Bilthoven, The Netherlands
[2]National Institute for Public Health and the Environment, Bilthoven; Department of Medical Microbiology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

In the last 3 years a series of surprising findings relating to the activity of PZA by multiple groups has been published. A low pH was assumed to be required for the activity of pyrazinoic acid (POA) against *M. tuberculosis*, the active from of pyrazinamide (PZA), but recently activity has been demonstrated at neutral pH. An increasing number of potential targets have been proposed for PZA in recent years raising the suspicion that PZA is a "dirty drug". However, it is our opinion that the recent demonstration that POA is active against PanD provides an alternative explanation for PZA's unusual activity. We propose PanD is the primary target of POA but expression of POA susceptibility requires an intact stress response. As the mycobacterial stress response requires the interaction of a number of genes, disruption

of any one could result in an inability to enter the sensitive phenotype. We believe this model can explain most of the recent observations of the seemingly diverse spectrum of activity of PZA. Solving the long standing puzzle and understanding the details of the true mechanism of action of PZA should allow new drugs also able to shorten treatment to be prioritised for development.

OP-20

Acetate mediated activation of DevR dormancy regulon in *Mycobacterium tuberculosis* by acetyl phosphate metabolic signal

Saurabh Sharma[1], Priyanka Kumari[1], Atul Vashist[1], Chanchal Kumar[1], Malobi Nandi[1], Jaya Sivaswami Tyagi[1]
[1]Department of Biotechnology, All India Institute of Medical Sciences, Ansari Nagar, New Delhi, India

Two-component signal transduction systems (TCS) are extensively used by Mtb for adaptation to stresses for its survival. Among them, DevRS/Rv2027c (DosRS/DosT) is one of the best-characterized TCS, which is essential for bacterial survival under hypoxia, a prevailing stress within granulomas. The conventional activation pathway involves phosphorylation of DevR by DevS/DosT histidine kinase(s) in presence of an inducing stimulus such as hypoxia. Nature has kept the scope for alternative mechanisms of response regulators activation. One such recently reported mechanism is DevR overexpression, which bypasses the requirement for its phosphorylation. During infection, infected guinea pigs are reported to accumulate organic acids, including acetate, within granulomas. Using sensor kinase knock out mutant strains, we show that Mtb generates acetyl phosphate (AcP) during aerobic growth on acetate and activates DevR regulon expression. AcP is generated by utilizing the AckA-Pta pathway and by perturbing the pathway in Pta overexpressing strain, we confirm that AcP levels determine DevR regulon expression. Our data provide evidence for an alternate activation pathway of DevR by a metabolic signal AcP. By using a H395Q kinase mutant of DevS, we further establish that in bacteria exposed to dual stresses of acetate and hypoxia, conditions that mimic granuloma environment, DevR is activated through two routes of phosphorylation of DevR, one through sensor kinases and other through AcP mediated phosphorylation. This in vivo study is a first report in *Mycobacterium tuberculosis* highlighting the relevance of metabolic signal in enabling AcP based activation of response regulator. It is finally concluded based on these findings that targeting DevR and not DevS/T is ideal for intercepting DevRST signaling cascade.

OP-21

Recombinant BCG expressing ESX-1 of *M. marinum* combines low virulence with cytosolic immune signaling and improved tuberculosis protection

Matthias Groeschel[1], Fadel Sayes[2], Sang-Nae Cho[3], Laleh Majlessi[2], Roland Brosch[2]
[1]Institut Pasteur, Paris, France; Research Center Borstel, Germany
[2]Institut Pasteur, Paris, France
[3]Department of Microbiology, Institute for Immunology and Immunological Diseases, Yonsei University College of Medicine, Seoul

Objectives: A feature of the only licensed TB vaccine BCG is the partial deletion of the ESX-1 type VII secretion system, which governs phagosomal rupture and cytosolic pattern recognition, key intracellular phenotypes linked to increased immune signaling. Our objective here was to improve protective efficacy by equipping BCG with the ESX-1 dependent phenotype of cytosolic access.
Methods: BCG was transformed with a vector containing the *esx-1* region of *Mycobacterium marinum.* BCG::ESX1^{Mmar} functionality was assessed by ESX-1 specific T-cell hybridomas. THP-1 wild-type and cGas/STING K.O. cells were used to study phagosomal access and activation of cytosolic nucleotide sensors. BALB/c, C57BL/6 and SCID mice were vaccinated to characterize cellular immunity and virulence. Independent mouse vaccination models at two different institutes were employed to study virulence and vaccine efficacy.
Results: This new ESX-1 proficient BCG can access the host cytosol and activates the cGas/STING/TBK1/IRF-3/type I interferon axis and AIM2-mediated NLRP3 inflammasome activity while maintaining low virulence. This results in both higher proportions of CD8+ T cell effectors against mycobacterial antigens shared with BCG and polyfunctional CD4+ Th1 cells specific to ESX-1 antigens. Importantly, independent mouse vaccination models show BCG::ESX-1^{Mmar} confers superior protection relative to parental BCG against challenges with highly virulent M. tuberculosis.
Conclusions: We describe the virulence-neutral expression of the ESX-1 type VII secretion system of *Mycobacterium marinum* in BCG. The functioning ESX-1 system enables this novel

vaccine candidate to rupture the phagosome and to induce cytosolic pattern recognition and dedicated innate immune signaling in mice, resulting in increased protection against tuberculosis.

OP-22

Whole Genome Sequencing as a Tool to Quantify Local Tuberculosis Transmission in British Columbia, Canada

Jennifer Guthrie[1], Clare Kong[2], David Roth[2], Mabel Rodrigues[2], Timothy Walker[3], Dona Foster[4], Patrick Tang[2], Victoria Cook[2], James Johnston[2], Jennifer Gardy[5]
[1]School of Population and Public Health, University of British Columbia, Vancouver, Canada
[2]British Columbia Centre for Disease Control, Vancouver, Canada
[3]Department of Microbiology and Infectious Diseases, Level 7, John Radcliffe Hospital, Oxford, United Kingdom
[4]Nuffield Department of Medicine, John Radcliffe Hospital, Oxford, United Kingdom
[5]School of Population and Public Health, University of British Columbia; British Columbia Centre for Disease Control, Vancouver, Canada

Whole genome sequencing (WGS) provides a high-resolution view of TB transmission, superior to genotyping methods. To quantify the burden of TB resulting from local transmission and to improve our understanding of the population structure and transmission in British Columbia, Canada, we conducted a population-based 10-year retrospective study.
A total of 2,290 clinical *Mycobacterium tuberculosis* (*Mtb*) isolates collected in BC (2005–2014), representing 99.3% of all culture-positive cases diagnosed in this period, were first genotyped by 24-locus MIRU-VNTR. The 974 (42%) clustered isolates (≥2 with identical MIRU-VNTR) and 247 isolates of special interest, such as drug-resistant isolates and serial isolates from individual patients, were directed to WGS using the Illumina HiSeqX and analysed with a bioinformatics pipeline developed by Oxford and Public Health England. Genotype and WGS results were linked to case-level clinical and demographic data. The proportion of isolates clustered decreased from 42% using MIRU-VNTR to 26% with WGS using a 20-SNP threshold, and to 24% using a 12-SNP threshold. Among foreign-born persons, only 7.7% of cases clustered by WGS, and transmission in this group was often within households. In contrast, the majority (77%) of Canadian-born TB cases did in fact represent local transmission, with 11 large outbreaks (11–72 cases/cluster) identified. Although WGS data suggested a significant degree of geographic structure to transmission, we detected individual transmission-events across large distances (>1,000 km).
Linking WGS results to case-level clinical and demographic data significantly enhances our understanding of the spread of tuberculosis in BC and will ultimately improve TB contact investigation efforts and permit effective allocation of resources.

OP-23

The relationship between transmission time and clustering methods *in Mycobacterium tuberculosis* epidemiology

Conor Meehan[1], Pieter Moris[2], Thomas Kohl[3], Jūlija Pečerska[4], Suriya Akter[1], Matthias Merker[3], Christian Utpatel[3], Patrick Beckert[3], Florian Gehre[5], Pauline Lempens[1], Tanja Stadler[6], Michel Kaswa[7], Denise Kühnert[8], Stefan Niemann[3], Bouke De Jong[9]
[1]Institute of Tropical Medicine, Antwerp, Belgium
[2]University of Antwerp, Belgium
[3]Molecular and Experimental Mycobacteriology, Research Center Borstel, Germany
[4]ETH Zürich, Switzerland
[5]Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany
[6]Computational Evolution, Biosystems Science and Engineering; ETH Zürich, Switzerland
[7]National Tuberculosis Program, Kingshasa, Congo
[8]Max Planck Institute for the Science of Human History, Jena, Germany
Institute of Tropical Medicine, Antwerp, Belgium

Tracking recent transmission is a vital part of controlling widespread pathogens such as *Mycobacterium tuberculosis*. Multiple approaches exist for detecting recent transmission chains, usually by clustering strains based on the similarity of their genotyping results. However, each method gives varying estimates of transmission cluster sizes and inferring when transmission events within these clusters occurred is almost impossible.
This study combines whole genome sequence (WGS) data derived from a high endemic setting (Kinshasa, Democratic Republic of

Congo) with phylodynamics to unveil the timing of transmission events posited by a variety of standard genotyping methods. Our results suggest that clusters based on spoligotyping could encompass transmission events that occurred hundreds of years prior to sampling while 24-loci-MIRU-VNTR often represented decades of transmission. Instead, WGS based genotyping applying low SNP thresholds allows for estimation of recent transmission events.
These findings can guide the selection of appropriate clustering methods for uncovering relevant transmission chains within a given time-period.

OP-24

OP-24
Transmission dynamics within a major *Mycobacterium tuberculosis* cluster in the Danish Kingdom spanning 23 years: What does whole genome sequencing add?

Dorte B. Folkvardsen[1], Anders Norman[2], Erik Michael Rasmussen[1], Lars Jelsbak[3], Åse Bengaard Andersen[4], Troels Lillebaek[1]
[1]International Reference Laboratory of Mycobacteriology, Statens Serum Institut, Copenhagen, Denmark
[2]Statens Serum Institut, Copenhagen, Denmark
[3]Department of Systems Biology, Dept. of Biotechnology and Biomedicine, Technical University of Denmark
[4]Department of Infectious Diseases, Rigshospitalet, Denmark

Background: Denmark, a TB-low burden country, experiences continued problems with active *M. tuberculosis* (*Mtb*) transmission and currently hosts Scandinavia's biggest clonal outbreak, termed Cluster 2 (C2), with over a thousand reported cases since 1992. The conventional genotyping methods for *Mtb*, RFLP and MIRU-VNTR, have been shown to have significant limitations when it comes to deciphering clonal outbreaks the size of C2. However, whole genome sequencing (WGS) with its higher resolution, can infer the direction of *Mtb* transmission with much greater accuracy.
Methods: We investigated a total of 951 whole genome sequenced C2-isolates, collected from 891 patients 1992 - 2014. This dataset constitutes 97% of all *Mtb* isolates identified as C2 in the Danish IRLM strain collection through RFLP or MIRU-VNTR during the study period. Instead of *Mtb* H37Rv, we used a completed genome of a C2-representative as the mapping reference, which increased the number of usable SNPs by 10%. We used a clustering criterion of ≤ 7 single-nucleotide polymorphisms (SNPs) between any two isolates, reflecting the maximum number of SNPs possible over 23 years at the calculated mutation rate of 0.2-0.3 SNPs / year, to identify possibly linked cases (including latent TB), and maximum likelihood (ML) phylogeny as well as median-joining network analysis to investigate the C2 outbreak in detail.
Results: Ninety-nine percent of all C2 strains were distributed into three previously identified epidemic groups (A, B & C) via single-linkage clustering. Furthermore, based on ML-phylogeny, 79% of isolates could be assigned to either of 13 discrete subgroups (A.1-A.11 or B.1-B.2), via one or more signature SNPs. Remarkably, more than half (*n*=529; 56%) of strains had no SNP differences to their nearest neighbor, even after undergoing at least one human-to-human transmission event. Using network analysis, we also demonstrate that signature SNPs correctly identify localized transmission chains throughout the Danish Kingdom.
Conclusions: Due to its higher resolution, WGS is helpful for ruling out person-to-person transmission, but the previously suggested thresholds of ≤ 5 SNP difference to infer direct transmission is ineffective for an outbreak of this large size in a limited geographical setting. Our data shows that signature SNPs and phylogenetic analysis are necessary tools for identifying and tracking discrete transmission chains within large on-going clonal outbreaks.

OP-25

Utility of whole genome sequencing (WGS) in practise

Rana Jajou[1], Han de Neeling[1], Arnout Mulder[1], Rina de Zwaan[1], Wim Van der Hoek[1], Richard Anthony[1], Dick van Soolingen[4]
[1]National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands
[4]National Institute for Public Health and the Environment (RIVM), Bilthoven; Department of Medical Microbiology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

Since 2016, all culture positive *M. tuberculosis* complex isolates have been subjected to WGS to allow comparison with current laboratory techniques in the Netherlands. The utility of WGS was investigated for identification of (sub) species and genotype families of the *M. tuberculosis* complex; for drug susceptibility testing; and to investigate transmission between tuberculosis patients.

For the identification of (sub) species and genotypes, a training set comprising 323 sequences, covering all (sub) species and genotypes, from the RIVM/the Netherlands and Oxford University/the UK were analysed and this resulted in the novel SNP-IT (SNPs to Identify TB) method. This method is based on the detection of SNPs shared exclusively by members of each (sub) species, lineage, and sub-lineage. The total number of unique SNPs identified ranged from 23 for *M. bovis* to 6,837 for *M. canettii*. The SNP-IT method was tested in a validation set comprising 614 sequences and was thereafter successfully applied to 3,128 clinical isolates.
For the prediction of drug susceptibility, phenotypic drug susceptibility testing (MGIT) was compared with the detection of resistance-associated mutations by WGS for the first-line antibiotics rifampicin, isoniazid, ethambutol, and pyrazinamide. In total, 1,134 isolates cultured in 2016/2017 in the Netherlands were included. For all drugs, the negative predictive value was >99% (100% for rifampicin). WGS was also able to predict intermediate/low level resistance for rifampicin, isoniazid, and pyrazinamide.
Finally, a population-based study was performed on all culture positive isolates received at the RIVM in 2016. Both VNTR typing and WGS were performed on all isolates. Isolates were clustered by VNTR if they shared identical 24-loci VNTR patterns; isolates were assigned to a WGS cluster when the pair-wise genetic distance was ≤ 12 SNPs. Cluster investigation was performed by municipal health services on all isolates clustered by VNTR in 2016. In total, 535 isolates were genotyped, of which 25% (134/535) were clustered by VNTR and 14% (76/535) by WGS; the concordance between both typing methods was 86%. The proportion of identified epidemiological links among WGS clustered cases (57%) was twice as high as the proportion of identified epidemiological links among VNTR clustered cases (31%). Thus, while WGS clustered half of the number of isolates, all epidemiologically linked cases remained clustered. Based on these analyses, WGS is ready to replace current identification tools, to prevent most of the resistance testing, and to serve as a more reliable epidemiological marker.

OP-26

Multidrug resistant *Mycobacterium tuberculosis* strains in Saudi Arbia: Myth, reality and worldwide impact

Hawra Al-Ghafli[1], Thomas Kohl[2], Bright Varghese[1], Matthias Merker[2], Stefan Niemann[2], Sahal Al-Hajoj Al-Nakhli[1]
[1]King Faisal Specialist Hospital and Research Centre (KFSHRC), Saudi Arabia
[2]Molecular and Experimental Mycobacteriology, Research Center Borstel, Germany

Several studies documented that Saudi Arabia is suffering from high level of Multidrug resistant *Mycobacterium tuberculosis* complex (MDR-MTBC) strains. Some of these studies even reported 44% MDR-TB rate, other studies showed variation of MDR-TB within different cities. However, as per our nationwide prospective study, guided by the World Health Organization, we found that the country is not suffering from high level of drug resistance. We consider this finding as the first reality, as all previous studies were small, retrospective, fragmented, not guided and not nationwide. The second reality is that the country is suffering from an ongoing transmission. This is evidenced by high cluster rates obtained upon genotyping more than 4000 isolates. This ongoing transmission is caused by unmatched huge clade diversity. Thereby, cross-border transmission of MDR-MTBC strains might be particularly fostered by high immigration influx and mobility dynamics. In an exploratory study, we investigated 71 MDR-MTBC strains collected from provincial mycobacteria referral laboratories in Saudi Arabia and compared demographic and clinical parameters to a convenient cohort of non- MDR-TB patients using whole genome sequencing approach. This guided us to third reality which indicates that the country is suffering from persistence of MDR-MTBC strains. It is worth mentioning that some patients are re-infected with MDR-MTBC strains. Persistence of MDR-MTB strains may lead to the development of extensively drug resistant strains. 22•5% of enrolled isolates were already predicted to be fully resistant to all five first-line drugs and (7•0%) exhibited fluoroquinolone resistance conferring mutations. Last but not least, our accumulated data showed that the nature of an ongoing transmission is admixing. This is totally opposite to the picture in Europe and elsewhere. Optimized TB molecular surveillance, diagnosis, and patient management are urgently needed to contain MDR-MTBC transmission and development of additional drug resistances.

OP-27

Using Whole Genome Sequencing to Decode *Mycobacterium tuberculosis* Transmission: application in a low-burden country

Irving Cancino[1], Yuanwei Xu[2], Luis Villamayor-Cebolla[3], Manuela Torres-Puente[1], Caroline Colijn[2], Iñaki Comas[4], Valencia Region Tuberculosis Working Group[5]
[1]Biomedicine Institute of Valencia (Ibv-Csic), Tuberculosis Genomics Unit, Valencia, Spain
[2]Department of Mathematics, Imperial College London, United Kingdom
[3]Genomics and Health Unit, Fisabio Public Heath, Valencia, Spain
[4]Biomedicine Institute of Valencia (Ibv-Csic), Tuberculosis Genomics Unit, Valencia, Spain; Department of Mathematics, Imperial College London, United Kingdom
[5]Valencia, Spain

With around 10 million new cases per year, tuberculosis (TB) is one of the most important infectious diseases in the world. Whole genome sequencing (WGS) is often used in TB surveillance programs due to its greater resolution compared to classical typing methods. Studies have demonstrated that WGS can identify outbreaks, predict transmission events and distinguish between relapses and co-infections. Despite this, the directionality of the transmission (whom infected whom) remains still uncertain. Here, we used WGS to identify, characterize and infer the transmission direction in a low-incidence country during a three-year period. Using pairwise nucleotide distances, we detected that 35-45% of the cases were clustered; clusters ranged from 2 to 12 isolates per group. In contrast, only 20% of the isolates were considered epidemiologically related by standard contact tracing. We applied network reconstruction analyses based only on genetic data to the larger clusters to predict transmission direction and missing cases. In parallel, we developed a model by combining epidemiological and phylogenetic data to obtain posterior estimates of the number of missing cases in the clusters. This study improves the understanding of how and when TB transmits. In addition, it highlights the relevance of WGS data for improving disease control.

OP-28

MTBseq: A comprehensive pipeline for whole genome sequence analysis of *Mycobacterium tuberculosis* complex isolates

Christian Utpatel[1], Thomas Kohl[1], Viola Schleusener[1], Maria Rosaria De Filippo[2], Patrick Beckert[3], Daniela Maria Cirillo[2], Stefan Niemann[3]
[1]Molecular and Experimental Mycobacteriology, Research Center Borstel, Germany
[2]Emerging Bacterial Pathogens Unit, Division of Immunology, Transplantation and Infectious Diseases, IRCCS San Raffaele Scientific Institute, Milan, Italy
[3]Molecular and Experimental Mycobacteriology, Research Center Borstel; German Center for Infection Research (DZIF), Partner Site Hamburg - Lübeck - Borstel – Riems, Germany

Developments in next generation sequencing (NGS) technologies allow the use of whole genome sequencing (WGS) as routine tool for bacterial strain characterization which lead to significant improvements for epidemiological surveillance of major pathogens such as *Mycobacterium tuberculosis* complex (MTBC). While the application of WGS clearly advances resistance prediction, in-depth genotyping, outbreak detection and, potentially, genomic surveillance of isolates MTBC isolates, no standard analysis pipeline allowing for local high quality data analysis has been proposed so far. Analyzing WGS data of MTBC isolates in a standardized workflow enables both, comprehensive antibiotic resistance profiling and outbreak surveillance, with highest resolution up to the identification of recent transmission chains. We therefore developed MTBseq, a customizable and expandable bioinformatics pipeline for local, offline next generation genome sequence data analysis of MTBC isolates. Employing a reference mapping based workflow, MTBseq reports detected variant positions annotated with known association to antibiotic resistance and performs a lineage classification based on phylogenetic single nucleotide polymorphisms (SNPs). When comparing multiple datasets, MTBseq provides a joint list of variants, SNP distance matrix, a FASTA alignment of SNP positions for use in phylogenomic analysis, and identifies groups of related isolates. We demonstrate the accuracy and sensitivity for resistance profiling, genotyping, and comparative analysis, concluding that MTBseq is a suitable automated solution for resistance deduction, phylogenetic classification and analysis of MTBC whole genome datasets.

OP-29

Recurrent tuberculosis in patients infected with a major *Mycobacterium tuberculosis* outbreak strain in Denmark. New insights gained through whole genome sequencing

Dorte B. Folkvardsen[1], Anders Norman[1], Erik Michael Rasmussen[1], Troels Lillebaek[1], Lars Jelsbak[2], Åse Bengaard Andersen[3]
[1]International Reference Laboratory of Mycobacteriology, Statens Serum Institut, Denmark
[2]Dept. of Biotechnology and Biomedicine, Technical University of Denmark
[3]Department of Infectious Diseases, Rigshospitalet, Denmark

Recurrent tuberculosis (TB), defined as a subsequent episode of TB after ended cure, is a big health problem and may be due to reinfection with a new strain or relapse with the old strain. In Denmark, a major outbreak of TB caused by one specific genotype documented with Restriction Fragment Length Polymorphisms or Mycobacterial Interspersed Repetitive Units – Variable Number of Tandem Repeats, is ongoing. During the period 1992 to 2014, 891 patients were infected with the genotype ascribed to be Cluster 2 or 1112-15 (C2) with either method. Of the 891 patients, 30 had one or more recurrent episodes, 63 episodes in total. None of the episodes were found to confer resistance to any of the four first-line-anti-TB drugs.
Whole genome sequencing (WGS) was applied to all cases of tuberculosis caused by the genotype C2 in the period. The 63 episodes from the 30 recurrent cases were compared in terms of SNP difference and time between episodes.
Two cases could be confirmed with relapse and one with reinfection. The remaining 27 cases could not be assigned to either or, even though they all had a SNP difference of <6 SNPs, which is proposed to confirm relapse. The average time passed between episodes in one patient is 4.1 years. Other studies indicate that reinfection with genetically similar strains are unlikely, and that for it to happen, longer passage of time between episodes must pass. We find recurrence with a highly genetically similar strain to be possible, even after a short time period.
Our findings show that even with the high resolution obtained with WGS, it can be very difficult to distinguish between relapse and reinfection, at least when dealing with at large outbreak. Performing phylogenetic analysis on the entire outbreak provides a more accurate distinction. The SNP algorithms proposed for distinguishing recurrent TB as relapse or reinfection using WGS in not valid in our setting. The high number of recurrent TB with the genotype C2 comparing to other *Mycobacterium tuberculosis* genotypes in Denmark might be attributed to extended virulence of the strain.

OP-30

Key role of mass migration, as opposite to ordinary human exchange, in global spread of M. tuberculosis strains

Igor Mokrousov[1]
[1]St. Petersburg Pasteur Institute, Russian Federation

Mycobacterium tuberculosis is a major human killer. Its most dangerous multidrug resistant strains emerge in the high-incidence world regions as a result of inadequate treatment and special pathobiological properties of these strains. Such strains are justly seen as global health concern and any world region is regarded to be at risk of their introduction and subsequent epidemic dissemination. The above said is a common cliché. The real situation is more nuanced.
I used as example two widespread phylogenetic lineages of *M. tuberculosis*, East-Asian (or Lineage 2) and Euro-American (or Lineage 4) and their most (and less) known genotypes and epidemic clones: Beijing, B0/W148, LAM, RD-Rio, LAM-RUS, Ural, NEW-1. I compiled and compared their phylogeographic and pathobiological patterns with recent events in human history and migration at regional and transcontinental level.
As a result of this analysis, I draw three interconnected conclusions about the role of human migration and demographics in the spread of emerging and epidemic strains of *M. tuberculosis*. First, ordinary human exchange/ travel is not enough to bring and settle down new *M. tuberculosis* strain in an autochthonous human population. In contrast, massive influx of migrants may change dramatically the population structure (human and pathogen's). Second, new emerging strain becomes emerging in its area of origin, where its parental strain was circulating. But not necessarily it will be successful and epidemic elsewhere, in genetically/ethnically different population. Third, to be efficiently imported to a new location, a strain should be sufficiently prevalent in its country of origin. In other words, a contagiosity of an *M. tuberculosis* strain is conditional, but not absolute feature. Speculatively, a kind of human resistance is developed in local population through its co-existence with historical local clones, and acting against imported clones: hence indirect evidence of the role of host genetics.
Funding: Russian Science Foundation grant 14-14-00292.

OP-31

Ecology of *Mycobacterium avium* group

Ivo Pavlik[1], Vít Ulmann[2], Jan Caha[1], Helena Modra[1], Ondrej Konecny[1], Dana Hübelova[1], Milan Bartos[3], Milan Gersl[4], Jan Kudelka[4]
[1]Faculty of Regional Development and International Studies, Mendel University Brno, Czech Republic
[2]Institute of Public Health Ostrava, Czech Republic
[3]Faculty of Science, Masaryk Univerzity in Brno, Czech Republic
[4]Faculty of Agriscience, Mendel Univerzity in Brno, Czech Republic

Introduction: *Mycobacterium avium* Group (*MAG*) represents an important part of species and subspecies of the *Mycobacterium* genus from epidemiological, epizootiological and ecological views. According to the currently valid taxonomy, the *M. avium* species is divided into four subspecies and *MAG* include 14 species. All these *MAG* members were isolates from clinical samples and except of *M. talmoniae* they were detected in different environmental matrices including amoebas. During last two decades, extended epidemiological and ecological studies were carried out in the Czech Republic (CR) concerning non-tuberculous mycobacteria (NTM) including *MAG* members.
Methodology: The prevalence of *MAG* members detected in 1,970 human patients in EU (1995–2011; van der Werf et al., 2014, BMC Infect. Dis. 14:62.) was compared with *MAG* members prevalence in 177 patients in the CR (1998–2017). From the published literature sources and current studies the *MAG* members prevalence in the environment (n=2,771) in the CR was analysed including space analyses. Hydrological, geochemical and other analysed parameters in different environmental matrices (surface water sediments from different water catchment areas, soil, mined peat and peatlands, wooden material, guano, earthworm castings, waste water treatment plants-WWTP sediments etc.) analyses were carried out.
Results: The *MAG* members' prevalence in EU and CR patients was similar. From 284 drinking water isolates from the CR 270 (95.0%) were identified as *M. a. hominissuis* and only 14 (5.0%) belonged to *M. intracellulare*. The potential risks of mycobacterioses caused by NTM and *MAG* members are according to published data in the CR in drinking water (18.8%), sediments from ponds' (26.4%), water reservoir (29.3%) and river water (14.1%), mined peat including potting soil (60.8%) and wooden material, especially sawdust (38.4%). Real time qPCR (whole genus *Mycobacterium*, IS*1245*, IS*901* and IS*900*) and NGS (next generation sequencing) demonstrated the role of genus *Mycobacterium* and *MAG* members in environmental microbiomes in WWTP sediments, guano, and earthworm castings and in surface water sediments.
Conclusions: Analysed data confirmed relatively high *MAG* members prevalence except of drinking water also in other matrices (water sediments, mined peat, sawdust etc.) than drinking water.
Acknowledgements: This work was supported by the Czech Science Foundation (Grant No. 16-13231S).

OP-32

Mycobacterial infections in Moravia and Silesia (Czech Republic) region during the years 2012-2016

Vít Ulmann[1], Jan Caha[2], Helena Modra[2], Ivo Pavlik[2]
[1]Institute of Public Health Ostrava, Czech Republic
[2]Faculty of Regional Development and International Studies, Mendel University Brno, Czech Republic

Introduction: Mycobacterioses represent serious health risk especially in immunosuppressed patients. Polluted working environment and unhealthy lifestyle increase the predisposition especially for lung mycobacterioses. The aim of the study was the prevalence evaluation of clinically related non-tuberculous mycobacteria (NTM) in Moravia (22 349 km²) and Silesia (4 459 km²) Regions. **Methodology:** During the studied 5 year period 54,625 clinical samples were examined by conventional and metabolic cultures. Isolates were identified by molecular biology methods (HainLifescience – GenoType Mycobacterium CM/AS and 16S DNA sequencing) and spatiotemporal analysis was done. Clinic pathological relevancies were evaluated according to national and ATS standard, microbiological a clinical findings.
Results: NTM were isolated from 1,842 samples: a total of 32 mycobacterial species

(1,390 isolates) were detected. Clinically relevant isolates originated from 208 (11.3%) patients. Lung infection predominated (196; 94.2%). *M. avium* was the most common isolated species; (63; 30%) *M. kansasii* (63; 30%) was followed by *M. xenopi* (44; 21%) and *M. intracellulare* (16; 7.7%). In 5 patients *M. malmoense* was diagnosed since 2013. Sporadic lung infections were caused by *M. colombiense*, *M. szulgai*, *M. fortuitum*, *M. mucogenicum*, *M. chelonae* and *M. abscessus*. Extrapulmonnary infections (12 patients; 5.8 %) were diagnosed in neck lymph nodes (*M. avium* in 5 patients and *M. intracellulare* in 1 patient), skin and ocular infections were caused by *M. chelonae* (2 patients), *M. marinum* (2 patients), *M. avium* (1 patients) and *M. vulneris* (1 patients). Any isolates of *M. gordonae* (283) were evaluated as clinically relevant, including the most prevalent *M. xenopi* (634 isolates), *M. fortuitum* (157 isolates), *M. smegmatis* (136 isolates), *M. celatum* (14 isolates), *M. goodii* (12 isolates) and *M. terrae* complex (*M. terrae*, *M. triviale*, *M. arupense* and *M. kumamotonense*). Spatiotemporal analysis confirmed previously observed *M. kansasii* patients' accumulation in the North part of the region in opposite to *M. avium* infections spread regularly in the whole region. **Conclusions:** Analysed data confirmed relatively high mycobacterioses prevalence in the studied region caused by intensive coal mining and heavy industry activities.

Acknowledgements: This work was supported by the Czech Science Foundation (Grant No. 16-13231S).

ABSTRACTS OF POSTER PRESENTATIONS (P)

Host-pathogen interaction

P 112

Detailed analysis of the infection of the same MDR *Mycobacterium tuberculosis* strain in two different patients: alone or in a confection with a susceptible strain

Estefanía Abascal[1], Marta Herranz[2], Fermín Acosta[1], Juan Agapito[3], María Jesús Ruiz Serrano[2], Francisco Fernández-González[1], Paloma Gijón[1], Pilar Gómez Pintado[4], Enrique Acín[4], Tatiana Cáceres[3], Patricia Muñoz[2], Eduardo Gotuzzo[3], Darío García de Viedma[2]

[1]Hospital General Universitario Gregorio Marañón, Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, Spain

[2]Hospital General Universitario Gregorio Marañón, Instituto de Investigación Sanitaria Gregorio Marañón, Madrid; CIBER Enfermedades Respiratorias (Ciberes), Spain

[3]Tb Research Unit, Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru

[4]General Subdirection of Penitentiary Health – Penitentiary Institutions – Ministry of Interior of Spain

Background: Mixed infections by two *Mycobacterium tuberculosis* strains challenge diagnosis and treatment of tuberculosis (TB), especially when different susceptibility patterns are involved. Despite these modalities of infection are assumed to be restricted to high burden contexts, international movements make more likely to face them also in low burden settings. We diagnosed in Madrid two cases, inmates coming from the same prison in Peru, infected by the same MDR strain. However, they represented two different infective dynamics, due to coinfection or not with another susceptible strain.

Materials/methods: MIRU-VNTR was performed on cultured isolates and on single colonies. GeneXpert-MTB/RIF, Anyplex™II-MTB/MDR/XDR and MGIT-SIRE and second line susceptibility tests were applied for the analysis of resistances. Whole Genome Sequencing (WGS) was applied for an in-depth analysis of the isolates.

Results: Two asymptomatic VIH- inmates, transferred independently from the same Peruvian prison to complete their sentence in Spain, were diagnosed of pulmonary TB at prison reception. MIRU-VNTR analysis from Case 1 revealed a mixed infection with two strains, both in sputum and stool, whereas case 2 was infected with a single strain. The analysis of single colonies from case 1 revealed the coinfection with a MDR and a pansusceptible strain at a ratio 7:3. Whole-genome sequencing confirmed that the MDR isolates from the two inmates corresponded to the same strain. Cases 1 and 2 received a similar anti-MDR-TB regimen and adherence was confirmed. However, case 1 extended his positivity period along almost 5 months of therapy whereas in case 2, cultures were negative after 1 month and until end of treatment. The analysis by MIRU-VNTR of the two coinfecting strains in case 1 along 14 sequential isolates indicated a prolonged persistence of the susceptible strain, even in the latest cultures analysed, when the MDR strain turned undetectable.

Conclusions: We must be alert about the importation of MDR-TB from high burden contexts and consider the possibility of clonal complexity in these cases. We describe how the same MDR strain, under equivalent anti-MDR therapy, offered different infective behaviours in two hosts. The persistence of a susceptible strain under anti-MDR treatment leads to discuss which should be the proper therapeutical management of this kind of clonally complex infections.

Funding: ERANET-LAC (TRANS-TB-TRANS; ELAC2015/T08-0664, E035-ERAnet-LAC/J110-2016/FONDECYT), ISCIII: (AC16/00057, FIS15/01554), IFARHU-SENACYT (270-2016-293) to F.A.

P 113

Intra-host Genetic Variability of *Mycobacterium tuberculosis* in a Patient with Pansusceptible tuberculosis

Marie Seraphin[1], Erik Michael Rasmussen[2], Anders Norman[2], Alexandra Gerace[1], Troels Lillebaek[2], Michael Lauzardo[1]

[1]University of Florida, Gainesville, United States

[2]International Reference Laboratory of

Mycobacteriology, Statens Serum Institut, Copenhagen, Denmark

Introduction: Whole genome sequencing (WGS) based studies have exposed a breadth of genetic diversity within *Mycobacterium tuberculosis*. In particular, short-term evolution, i.e. microevolution, potentially due to a faster *in vivo* mutation rate is hypothesized to drive within-host variation during clinical infection. In these analyses, we investigate the influence of drug pressure on the within-host dynamic of *M. tuberculosis* using serial isolates from a TB case undergoing standard combination therapy. Pre-treatment isolates from the case and his epidemiologically confirmed secondary case were available, allowing us to compare the dynamic of within-host evolution to pre-treatment states of *M. tuberculosis*.

Methods: In addition to the pre-treatment isolates, 11 within-host isolates were collected over two months of therapy. We divided the sampling period into four biweekly intervals with at least 2 within-host isolates collected within each interval. We sequenced 13 illumina libraries using the MiSeq platform. We mapped reads to the H37Rv reference strain at an average depth of 86.4 x and 97.7% coverage. We inferred the phylogenetic relationship using distance and maximum likelihood based methods. We annotated the reassembled genomes to investigate the functional consequences of the SNPs identified. Using a minimum frequency of 85% to consider a SNP fixed and a maximum p-value of 0.005 for low frequency SNPs we tracked the dynamic of different SNPs across the sampling intervals.

Results: The 13 genomes were phylogenetically identical, with an average of one SNP difference between them. Pairwise SNP comparisons revealed no genetic variation among the serially samples within-host genomes and the two pre-treatment genomes from days 0 to 30 of therapy, supporting the hypothesis that variation accumulated later on, possibly as a result of drug pressure. We identified seven different SNPs at varying frequencies across the four sampling intervals. At each biweekly interval, genetic variation seemed to accumulate steadily to fixation and then decrease in frequency at subsequent interval. Of the seven SNPs, six were in CDS with four leading to an amino acid change.

Conclusion: Under the influence of treatment effect, within-host diversity is driven by genetic drift. Further analyses employing deep sequencing are needed to confirm these findings.

P 115

Role of the Cholinergic System in Experimental Pulmonary Tuberculosis

Leon Islas-Weinstein[1], Jorge Barrios-Payán[2], Dulce Mata-Espinosa[2], Brenda Marquina-Castillo[2], Mario Alberto Zetter-Salmón[1], Rogelio Hernández-Pando[1], Iris Selene Paredes González[1], Octavio Ramos-Espinosa[1]

[1]Experimental Pathology Section, National Institute of Medical Sciences and Nutrition, Mexico City; PhD Program in Biomedical Sciences, National Autonomous University of Mexico (UNAM)

[2]Experimental Pathology Section, National Institute of Medical Sciences and Nutrition, Mexico City

The cholinergic system (CS) is responsible for coordinating the synthesis, actions and degradation of acetylcholine (ACh), an endogenous nicotinic receptor agonist synthesized by neuronal and non-neuronal cells in mammals. In the airways, ACh alters the recruitment of immune cells and inhibits cell mediated immunity mainly by blocking the nuclear translocation of NFκB. Additionally, evidence has accumulated proving that ACh is an evolutionarily ancient molecule and may be produced by prokaryotic and eukaryotic organisms belonging to distinct kingdoms of life including bacteria, archaea, plantae and fungi. Due to the presence of the CS in both bacteria and the airways, we determined if it played a role in the pathological progression of pulmonary tuberculosis (TB). TB is the infectious disease with more attributed deaths worldwide. Using an ELISA assay, ACh production by *Mycobacterium tuberculosis* (Mtb) was demonstrated during different growth phases of the bacteria. Using a minimum stimulatory growth assay, (Mtb) increased its growth rate after the addition of nanomolar quantities of ACh. Conversely, the selective nicotinic ACh receptor (nAChR) antagonists dihydro-beta-erythroidine and methyllycaconitine possess bactericidal activity. Lungs from BALB/c mice infected with Mycobacterium tuberculosis (Mtb) H37Rv reveled increased ACh concentrations during specific periods of disease progression. During these periods of increased lung ACh, mice were treated with saline solution (control group) or with the two nAChR selective antagonists. Mice lungs treated with the nAChR antagonists had improved disease parameters (colony forming units and lung inflammation) compared to the control group. Thus, the CS may emerge as a novel therapeutic target in treating TB infection, improving the host's response during experimental pulmonary tuberculosis.

P 117

Immunoinformatics approaches to investigate to molecular basis underlying varying adaptive immune responses induced by different *Mycobacterium tuberculosis* lineages

Carlos Magalhães[1], Iñaki Comas[2], Margarida Saraiva[3], Nuno Osório[1]
[1]Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Campus Gualtar; ICVS/3b's - Pt Government Associate Laboratory, Braga/ Guimarães, Portugal
[2]Ciber En Epidemiología Y Salud Pública; Instituto de Biomedicina, IBV-CSIC, Valencia, Spain
[3]I3s - Instituto de Investigação e Inovação Em Saúde; IBMC - Instituto de Biologia Molecular e Celular, Universidade Do Porto, Porto, Portugal

Tuberculosis (TB) is the deadliest infectious disease in the history of humankind and remains vastly uncontrolled. Active TB can result from infection with distinct genetic lineages of the *Mycobacterium tuberculosis* complex with lineages 1, 2 and 4 being the most frequent. Interestingly, a strong geographic association between active TB cases and specific lineages exists, which seems to be disrupted in the context of HIV-1 co-infection. This fact highlights the relevance of CD4+ T cell-driven immune responses in the interaction between different human/pathogen populations and TB outcome. CD4+ T cells recognize peptides (epitopes) bound to histocompatibility leukocyte antigen (HLA) class II proteins, a step required for CD4+ T cell differentiation and function. *M. tuberculosis* lineage-specific epitope diversity might thus alter the type and level of CD4+ T cell responses generated during infection. Despite the importance of this topic, host-pathogen molecular characteristics influencing the immune synapse in TB are not sufficiently studied. We have developed a genome-wide immunoinformatics approach to predict T cell epitopes that are statistically influenced by the presence of *M. tuberculosis* lineage-specific polymorphisms. We have preliminary data supporting the existence of lineage-restricted CD4+ T cell epitopes. Importantly, in some cases, it was possible to find a significant association between the HLAs binding predictions for a given lineage and the frequency of the HLAs in the human populations with more TB caused by that lineage. Some mutant epitopes were also inferred to have been selected over time by distinct computational molecular evolution methodologies. Overall, this study suggests that specific *M. tuberculosis* lineage-specific polymorphisms have been fixed during parallel evolution with the host due to CD4+ T cell pressure. The identification and extensive characterization of varying *M.tuberculosis* epitopes might be of great relevance for the development of more effective TB vaccination and diagnostics strategies.

P 135

Buruli ulcer disease by *Mycobacterium ulcerans* in Mexican pediatric patient

Virginia Lora-Téllez[1], María Lucía Pérez-Ricardez[1], María Fabiola Lara-Hernández[1], María Concepción Mella-Romero[1], Rigoberto Hernández-Castro[2], Roberto Arenas-Guzmán[2]
[1]Hospital Para el Niño Poblano, Puebla, Mexico

Introduction: Buruli ulcer is an infectious disease involving the skin, caused by *Mycobacterium ulcerans,* characterized by a painless nodule, papule, plaque or oedema, evolving into a painless ulcer with undermined edges, often leading to invalidating sequelae. After tuberculosis and leprosy, Buruli ulcer is the third most common mycobacterial disease in immunocompetent humans. This disease has been reported from at least 27 countries around the world mostly in tropical or subtropical areas; and the children under the age of 15 years are predominantly affected without sex differences.
Confirmed clinical case description. Male child Mexican 13 years old, who lives in subtropical area at Mexico country, reports a papule located on right forearm, caused for an unknown insect 3 months ago, and the papule afterward evolved into a painless ulcer with undermined edges; with hyperplastic adjacent epidermis and necrotic slough. Several samples were collected by swab and biopsy for laboratory test. 1) Direct smear examination Ziehl-Neelsen stain showed many acid-fast bacilli; 2) *in vitro* culture of *Mycobacterium ulcerans* failure because a secondary bacterial infection non-mycobacterial; 3) histopathological examination showed adipose cells without nuclei, retain their cell wall and acid-fast bacilli inside. Many acid-fast bacilli were invaded the interstitium of the adipose tissue and lobar septa of the subcutaneous tissue; 4) identification of *Mycobacterium ulcerans* was positive by real-time PCR targeting the IS2404 sequence.
Both along 3 weeks Vacuum Assisted Closure therapy; and six months along with rifampin, streptomycin, clarithromycin antimicrobial therapy were success.

P 150

Genome Sequencing and comparative genomics of 12 Brazilian *Mycobacterium kansasii* clinical isolates

Edson Machado[1], Philip Noel Suffys[2], Luciana Destasio de Carvalho[3], Vinicius Mussi[4], Elena Lasunskaia[5], Conor Meehan[6], Antonio Basílio de Miranda[7], Marcos Catanho[1]
[1]***Laboratory for Functional Genomics and Bioinformatics, Oswaldo Cruz Institute, Fiocruz, Rio de Janeiro, Brazil***
[2]***Laboratory of Molecular Biology Applied to Mycobacteria, Oswaldo Cruz Institute, Fiocruz, Rio de Janeiro, Brazil; Insitute of Tropical Medicine in Antwerp, Belgium***
[3]***National Reference Center for Tuberculosis Prof Helio Fraga, Rio de Janeiro, Brazil***
[4]***Laboratory of Biology of Recognition, UENF, Brazil***
[5]***Universidade Estadual Do Norte Fluminense, Brazil***
[6]***Institute of Tropical Medicine, Antwerp, Belgium***
[7]***Laboratory of Computational and Systems Biology, Oswaldo Cruz Institute, Fiocruz, Rio de Janeiro, Brazil***

Introduction: Nontuberculous *Mycobacterium* (NTM) species are widespread in the (human-made) environment and some species cause opportunistic infections in humans. *Mycobacterium kansasii* is frequently isolated from tap water, is commonly isolated from patients with pre-existing lung disease, similar to other NTM clinically species. In Rio de Janeiro, *M. kansasii* is the most frequent NTM causing pulmonary disease.
Methods and results: The genome sequence of 12 clinical *M. kansasii* genotype I strains were isolated from human Brazilian patients with pulmonary disease, residents of the states of Rio de Janeiro (n=7), Pernambuco (n=3), Rio Grande do Sul (n=1) and Santa Catarina (n=1). Genomes were *de novo* assembled using SPAdes (GenBank accession numbers PQOL00000000-PQOW00000000), and automatically annotated applying two distinct pipelines: NCBI PGAP and RAST.
A reference-based SNP calling against the genome of the reference strain *M. kansasii* ATCC 12478 was performed with both Snippy and BioNumerics (wgSNP module), and showed a non-synonymous nucleotide substitution in codon 411 of gene rpoB for isolates 6498 and 8835, causing an amino acid change in their respective protein product in both isolates. In addition, these two isolates present a Minimal Inhibitory Concentration of > 1 µg/ml for rifampicin *in vitro* analysis.
Based on available literature, we constructed a catalog of 456 virulence factors of the widely studied *M. tuberculosis* H37Rv genome.
By applying the OMA stand-alone software we found 3524 orthologous genes between *M. kansasii* and *M. tuberculosis* H37Rv, of which 378 we defined as putative virulence associated genes in *M. kansasii* genome. The presence of SNPs in 180 putative virulence genes was verified in all 12 clinical isolates, including genes encoding RNA sigma factors, type VII secretion proteins, Mce family proteins, among others. These results are being compared with different *M. kansasii* virulence phenotypes *in vitro* and *in vivo* verified in a collaborate study, in which those 12 *M. kansasii* clinical isolates are being classified according to their distinct virulence profiles.
Conclusion: A considerable variability in both the genome composition (SNPs and large deletions) and *in vitro* and *in vivo* virulence was observed among the *M. kansasii* genotype I isolates from Brazil. We are currently analyzing gene absence and the expansion and/or reduction of gene families of different functional category. In addition, the presence of SNPs in putative virulence genes of these *M. kansasii* isolates is being compared with the virulence profile displayed by these pathogenes.

P 201

Rv3484, a LytR-cpsA-Psr protein encoding gene of *Mycobacterium tuberculosis*, is essential to establish infection in the mouse model of tuberculosis

Sven Malm[1], Silvia Maaß[1], Ulrich E. Schaible[1], Stefan Ehlers[2], Stefan Niemann[1]
[1]***Molecular and Experimental Mycobacteriology, Research Center Borstel, Germany***
[2]***Molecular Inflammation Medicine, Research Center Borstel, Germany***

LytR-cpsA-Psr (LCP) proteins have been described to play a role in cell wall biosynthesis. Four LCP proteins are present in members of the *Mycobacterium tuberculosis* complex (*Mtbc*) and functional redundancy of these proteins has initially been proposed. Here we describe the generation of a Rv3484 deletion mutant in *Mycobacterium tuberculosis* H37Rv. The loss of Rv3484 led to a severe attenuation of the bacteria in the mouse model of tuberculosis which could not be compensated by the other LCP proteins encoded by the *Mtbc*. Moreover we characterized the mutant strain in *in vitro* assays and found differential resistance to cell

wall targeting agents. Detailed analysis of the underlying mechanisms leading to the observed phenotype, also with respect to the recently proposed dual function of Rv3484 in cell wall biosynthesis and host-pathogen interaction, is objective of our current research.

P 6

Designing of a method based on reverse-hybridization line probe assay (LiPA) for rapid detection of prevalent Mycobacterium species from clinical specimens

Reza Kamali Kakhki[1], Ehsan Aryan[1], Mojtaba Sankian[2], Zahra Meshkat[1]
[1]*Antimicrobial Resistance Research Center, Mashhad University of Medical Sciences, Mashhad, Iran*
[2]*Immunobiochemistry Laboratory, Immunology Research Center, Bu-Ali Research Institute, Mashhad, Iran*

Background: Mycobacteria are the important pathogens for humans and have been emerged as a major health problem worldwide. In order to control the mycobacterial diseases, improved diagnostic tools are needed. Line probe assay is one of the strong tools for detection of different Mycobacterium species. There are two commercially available kits for the detection of *Mycobacterium spp*. based on line probe assay, named, the INNO-LiPA (Immunogenetics, Ghent, Belgium) and the GenoType Mycobacterium CM/AS system (Hain Life Science, Nehren, Germany) and have been approved by the FDA for use in cultivated specimens (not clinical specimens). However, rapid detection of *Mycobacterium spp*. directly from clinical specimens can be very valuable, because it obviates the need for time consuming process of cultivation.
The aim of this study was to develop a reverse hybridization line probe (LiPA) assay based on internal transcribed spacer (ITS) sequences for detection and differentiation of mycobacterial species or subspecies, and also evaluate the usefulness of LiPA for the detection of mycobacterial co-infections from clinical specimens.
Method: The analysis of polymorphism/conserved ITS sequences of 40 *Mycobacterium spp*. were performed using BioEdit software and BLAST program. The fifteen probes and one pair of primer based on ITS sequences were designed. The amplification of the target is performed using biotinylated primers. Then, labeled PCR products are hybridized with the oligonucleotide probes immobilized on strips. The specificity of the probes was evaluated using various strains of bacteria. The LiPA assay was validated using twenty reference strains belonging to thirteen *Mycobacterium spp*. Further evaluation of the LiPA was carried out on fifteen clinical isolates which had been identified as mycobacteria by PCR.
Results: The fifteen specific probes were used in this study hybridized only to sequences from the corresponding species. The fifteen clinical specimens were correctly identified by LiPA and confirmed by sequencing. Three of the fifteen clinical specimens showed co-infection with *M. tuberculosis* and non-tuberculosis mycobacteria.
Conclusions: The LiPA assay is an efficient tool for simultaneous detection of Mycobacterium genus and differentiation of various mycobacterial species. It could also be useful for the detection of co-infections in clinical specimens or mixed cultures. The rapid and direct identification of Mycobacterium could further reduce diagnostic time and thereby improve control and treatment of the diseases caused by *M. tuberculosis* or non-tuberculosis mycobacteria. So, it is suggested that the LiPA assay can be integrated into routine microbiological laboratory practice for diagnosis of mycobacterial infection especially for clinical specimens.

P 7

Using novel DNA target for the rapid detection of *Mycobacterium tuberculosis* from clinical samples

Reza Kamali Kakhki[1], Alireza Neshani[1], Mojtaba Sankian[2], Kiarash Ghazvini[1], Amin Houshyar[1], Mahsa Sayyadi[1]
[1]*Antimicrobial Resistance Research Center, Mashhad University of Medical Sciences, Mashhad, Iran*
[2]*Immunobiochemistry Laboratory, Immunology Research Center, Bu-Ali Research Institute, Mashhad, Iran*

Background: Rapid detection of tuberculosis is one of the important steps for control of the disease. Conventional methods like Lowenstein Jensen (LJ) culture and direct staining remain as cornerstone for detection of tuberculosis. However, these techniques are often time consuming and tedious, difficulty reading and interpreting the results, and some of them have low sensitivity and specificity. Molecular techniques can be both quicker and more sensitive than conventional methods and are useful tools for rapid diagnosis of the disease. PCR is one of these techniques

used in molecular laboratory. Various DNA targets have been used on the base of PCR method for detection of *M. tuberculosis complex* like *IS6110, mpb64, sdaA* and etc. Of them, *IS6110* has the highest sensitivity because multiple copies of the *IS6110* element usually exist in *M. tuberculosis*. Unfortunately, some *M. tuberculosis* isolates especially in Southeast Asia lack this element. The short-chain dehydrogenases/reductases gene (SDR) is a specific DNA sequence of *M. tuberculosis* and has not been used as DNA target for detection of *M. tuberculosis*.
The aim of this study was to assess the new target based on PCR method for rapid detection of *M. tuberculosis complex* from clinical samples.
Method: The primers were designed using the Primer Premier 5.0 software. The specificity evaluation of the primers was performed with computer-aided analysis using BLASTN. The specificity and sensitivity of the primers were checked by standard bacteria. A total of 100 clinical samples were evaluated by PCR.
Results: The SDR is a specific DNA sequence of *M. tuberculosis* and no amplification was observed with non-tuberculosis mycobacterium and other pathogenic bacteria. The designed primers were optimized and the detection limit was estimated. The PCR detection limit was 10fg of template DNA. The primers detected *M. tuberculosis* in the clinical samples with 87% sensitivity and 100% specificity.
Conclusions: The Characteristics of the SDR gene of *M. tuberculosis* include location on chromosomal gene (unlike *IS6110* gene) and presence in all cultivated samples used in this study, great detection limit (10fg) in the PCR-based detection and high overall specificity and sensitivity for detection of *M. tuberculosis complex* from clinical samples. Then, the SDR gene can be a useful target for detection of *M. tuberculosis* complex from clinical specimens.

P 15

TB-SeqDisK: a microfluidics platform in combination with whole genome sequencing for ultra-fast notification of *Mycobacterium tuberculosis* and its resistance pattern

Thomas Kohl[1], Markus Beutler[2], Matthias Merker[1], Tobias Paprotka[3], Kerstin Rönsch[3], Markus Schmitt[3], Wolfgang Grasse[4], Christoph Metzger-Boddien[4], Katharina Dormanns[5], Jan Lüddecke[5], Nils Paust[5], Judith Schlanderer[5], Lubov Delamotte[2], Jörg Schickedanz[6], Michael Steinwand[6], Stefan Niemann[1], Harald Hoffmann[2]
[1]Molecular and Experimental Mycobacteriology, Research Center Borstel, Germany
[2]IML Red GmbH, Germany
[3]GATC Biotech, Germany
[4]Gerbion GmbH & Co. KG, Germany
[5]Hahn-Schickard, Germany
[6]Qiagen Lake Constance GmbH, Germany

The emergence and spread of multidrug resistant (MDR, defined by resistances toward at least isoniazid and rifampicin) and extensively drug-resistant (XDR, defined by MDR plus resistance toward a fluoroquinolone and at least one second line injectable drug) strains of the *Mycobacterium tuberculosis* complex (MTBC) pose serious challenges to successful control of tuberculosis (TB) world-wide. Rapidly increasing numbers of MDR-TB cases question the prospects of the “End TB Strategy” formulated by the world health organization (WHO) in 2016, which pustulates a reduction of TB induced mortality by 95%, TB related incidence by 90%, and poverty due to TB by 100% until 2035.
In 2016, there were an estimated number of 600,000 new cases with rifampicin resistant or MDR tuberculosis, with 9.5% of these being XDR-TB. According to the WHO, treatment success-chances of those patients rank below 50%, particularly in high incidence countries where sub-optimal drug- and patient management can lead to long-term health complications and the risk of further spread of resistant TB strains in the community. Fast resistance profiling and early selection of effective treatment regimens is the key to rapidly inhibit TB-transmission, especially for resistant strains. To realize this, only a limited spectrum of tools is currently available. Since standard phenotypic drug susceptibility testing takes weeks, currently only PCR based tests provide fast results in routine diagnostics. However, PCR based assays only test for a very limited set of known resistance associated polymorphisms in the MTBC genome.
With TB-SeqDisK financed by the German Ministry of Research and Education, we aim to explore a solution that will combine fast testing for TB and major resistance markers within less than one hour, and determination of the complete resistance profile by whole genome sequencing within five days. Initial testing will cover resistance markers of both key drugs isoniazid and rifampicin. TB-SeqDisK uses a combination of microfluidic DNA preparation and the new geometric multiplexing technology in a disc platform which runs in a dedicated centrifuge cycler. The disc will also provide extracted DNA which will be applied to whole genome sequencing for the determination of the bacterial resistome, which will provide a strong advantage over the wide-spread Xpert MTB/RIF.

P 30

Direct detection of *Mycobacterium tuberculosis* rifampin resistance in bio-safe stained sputum smears

Surabhi Lavania[1], Divya Anthwal[2], Manpreet Bhalla[3], Nagendra Tomar[4], Sagarika Haldar[2], Jaya Tyagi[1]
[1]Department of Biotechnology, All India Institute of Medical Sciences, New Delhi, India
[2]Center for Bio-Design and Diagnostics, Translational Health Science and Technology Institute, NCR Biotech Science Cluster, Faridabad, India
[3]Department of Microbiology, National Institute of Tuberculosis and Respiratory Diseases, Mehrauli, New Delhi, India
[4]School of Biotechnology, Gautam Buddha University, Yamuna Express Way, Greater Noida, Uttar Pradesh, India

Inspite being less sensitive, direct smear microscopy of sputum forms the mainstay of TB diagnosis in high-burden settings. Stained sputum smear slides can serve as a ready-made resource to transport sputum for molecular drug susceptibility testing. However, bio-safety is a major concern during transport of sputum/stained slides and for laboratory workers engaged in processing *Mycobacterium tuberculosis* infected sputum specimens. In this study, a bio-safe USP concentration-based sputum processing method (Bio-safe method) was assessed on 87 *M. tuberculosis* culture positive sputum samples. Samples were processed for Ziehl-Neelsen (ZN) smear, liquid culture and DNA isolation. DNA isolated directly from sputum was subjected to an IS*6110* PCR assay. Both sputum DNA and DNA extracted from bio-safe ZN concentrated smear slides were subjected to *rpoB* PCR and simultaneously assessed by DNA sequencing for determining rifampin (RIF) resistance. All sputum samples were rendered sterile by Bio-safe method. Bio-safe smears exhibited a 5% increment in positivity over direct smear with a 14% increment in smear grade status. All samples were positive for IS*6110* and *rpoB* PCR. Thirty four percent samples were RIF resistant by *rpoB* PCR product sequencing. A 100% concordance (κ value=1) was obtained between sequencing results derived from bio-safe smear slides and bio-safe sputum. This study demonstrates that Bio-safe method can address safety issues associated with sputum processing, provide an efficient alternative to sample transport in the form of bio-safe stained concentrated smear slides and can also provide information on drug (RIF) resistance by direct DNA sequencing.

P 37

Pervasive effect of contaminant DNA on MTB whole genome sequencing outcomes: a study across clinical settings and sample specimens

Galo A. Goig-Serrano[1], Iñaki Comas[1]
[1]Tuberculosis Genomics Unit, Biomedicine Institute of Valencia, Spain

In recent years, a remarkable effort has been made to improve the whole genome sequencing (WGS) of *Mycobacterium tuberculosis* samples, from DNA extraction to high-throughput data analysis. As the technical complexity associated smoothes in terms of performance, time and decreasing costs, WGS will become a promising tool for routine diagnosis and real-time epidemiology investigations in a near future. Thus a thorough analysis of the data generated is critical to procure accurate diagnostics.
Sample contamination or presence of non-tuberculosis bacteria might be a very important factor to take into account when performing such analyses. Even in cases where WGS is done on pure, solid culture, contamination can be a major source of error, especially when predicting drug resistance.
Assessment of sample contamination is not always carried out and, even in publications where it is evaluated, there are no clear guidelines about how to manage those results. To our knowledge, no prior study has been conducted to assess the impact of sample contamination in the outcome of WGS analyses. In this study we evaluate this impact by analyzing more than 1700 samples from datasets representing a range of epidemiological settings and samples sequenced. We implement a comparative analysis pipeline to evaluate the effect of contamination by identifying and filtering out reads from contaminating taxa. Our results show that analyzing WGS data without cleaning non-tuberculosis reads can lead to inaccurate diagnostics in terms of drug susceptibility prediction and have a great impact in variant calling outcomes, not only for direct sequencing from clinical samples, but also for standard culture methods. Our work demonstrates the essential role of contamination assessment and cleaning steps in analysis pipelines if WGS is to be implemented as one of the main routine methods for the diagnosis of tuberculosis.

P 41

Integrating functional genomics and phylogenetics for the discovery of new antibiotic resistance determinants in *Mycobacterium tuberculosis*

Victoria Furió[1], Miguel Angel Moreno[1], Álvaro Chiner-Oms[2], Manuela Torres-Puente[1], Luis Villamayor[2], Iñaki Comas[1]
[1]Tuberculosis Genomics Unit, Biomedicine Institute of Valencia, Spain
[2]FISABIO Public Health

Isoniazid is one of the first-line antibiotics most widely used to treat tuberculosis, yet the set of known resistance-conferring mutations is incomplete, and we cannot always predict the phenotype. Studying isoniazid resistance in *Mycobacterium tuberculosis* is extremely complex, partly due to the impossibility to generate clinical resistance mutants in vitro. We can overcome this obstacle by using a functional genomics approach in which we systematically mutate every gene on the genome and observe the resulting effect. Transposon sequencing (Tn-seq) integrates massive transposon-insertion mutagenesis and next generation sequencing to evaluate the importance of genomic features in bacteria. Relative frequencies of the different mutants can be assessed by amplifying and sequencing transposon junctions. We used this technique to determine candidate resistance determinants of isoniazid in *Mycobacterium tuberculosis* H37Rv, obtaining a list of genomic features that alter growth in the presence of the antibiotic when disrupted. This list contained resistance genes already reported in the literature, as well as new regions. We then assessed their clinical relevance by performing a phylogenetic association test using 4763 globally distributed strains. This test showed that a subset of the candidates tend to cumulate mutations in resistant branches of the phylogeny, allowing us to explicitly associate them with resistance. Our results show that combining functional genomics and clinical data is a powerful approach to finding new resistance determinants for isoniazid. We are confident that this method can be extended to study other antibiotics, as well as to find potential drug resistance genes for new drugs.

P 57

Validation of the SensititreTM microbroth dilution method for *M. tuberculosis* complex (MTBC) antimicrobial susceptibility testing (AST)

Nurhazirah Mohd Ya'akob[1], Shuwei Zheng[1], Kee Mong Ha[1], Bin Eng Cynthia Chee[2], Yee Tang Wang[2], Li-Hwei Sng[1]
[1]Central Tuberculosis Laboratory, Singapore General Hospital, Singapore
[2]Tb Control Unit, Tan Tock Seng Hospital, Singapore

Background: The treatment and control of drug-resistant tuberculosis is a major dilemma globally. The Bactec960 MGIT (Becton Dickinson) provides accurate and reliable automated AST results and is the WHO-endorsed broth microdilution method. The advantages of the microbroth method include the ability to test a large panel of drugs and obtain more precise Minimal Inhibitory Concentrations (MICs) within a single test, providing resolution of discrepancies between single critical concentration AST and molecular testing; and obtaining AST results with rare failures of growth of MTC in the MGIT system. Here we present a validation study for using the Sensititre microbroth dilution method for MTBC AST.
Material / methods: Thirty eight clinical MTBC isolates which were resistant to at least one of the first-line anti-tuberculosis drugs by the MGIT method, and 1 pan-susceptible reference isolate, were tested by the microbroth method using MycoTB plates (Trek Diagnostic Systems, East Grinstead, UK) and the MICs were compared to the MGIT results. A subset of isolates was tested by the MGIT method for amikacin (n=18), moxifloxacin (n=19) and p-aminosalicylic acid (n=21); cycloserine and rifabutin were not tested. Discrepancies were resolved by retesting alternate isolates from the same patient or by the Hain MTBDR*plus* and *sl* tests (Hain Lifescience, Nehren, GmbH). Quality control strains H37Rv and H37Ra were used for performance verification of the testing process, in accordance to the Clinical and Laboratory Standards Institute's (CLSI) 3 x 5 process.
Results: There was 95 to 100% categorical agreement between the Sensititre microbroth and the MGIT macrobroth dilution methods for all drugs, except for ethambutol (84.6%) and ethionamide (81.1%). The discrepancies were largely due to isolates with MICs around the interpretive breakpoint for both methods. Otherwise, the essential agreement was 100% for all drugs except for ethionamide (91.9%) and isoniazid (97.4%). The overall agreement between 2 methods was excellent if the results

from the Hain test and retesting with the same or alternate isolates were taken into consideration.
Conclusion: The Sensititre microbroth dilution method using the MycoTB plate was found to be a suitable alternative method for MTBC AST and reliable in providing more precise MICs for guiding the treatment of drug-resistant patients.

P 75

Evaluation of Xpert MTB/RIF Ultra performance for pulmonary tuberculosis diagnosis on respiratory smear-negative samples in a French center

Elisabeth Hodille[1], Audrey Maisson[2], Laurine Charlet[2], Bauduin Clyde[2], Charlotte Genestet[3], Isabelle Fredenucci[1], Jean-Philippe Rasigade[1], Gérard Lina[4]
[1]***Microbiology Department, Hospices Civils de Lyon, Hôpital de la Croix Rousse, Lyon, France***
[2]***Centre Hospitalo-Universitaire de Lyon, France***
[3]***CIRI INSERM U1111, University of Lyon, France***
[4]***Hospices Civils de Lyon, Centre National de Référence des Staphylocoques, CIRI INSERM U1111, France***

Background: Tuberculosis (TB) is a worldwide public health concern, including in high-resource countries with a low TB incidence. Xpert MTB/RIF assay was developed to improve TB and rifampicin-resistance detection, but the sensitivity remains weak on smear-negative sputum (between 28% and 67% according to studies). Xpert MTB/RIF Ultra assay has been designed to enhance the overall sensitivity of TB detection in clinical samples. Here, we evaluated retrospectively the performance of this test on smear-negative respiratory samples.
Materials/methods: Forty-six respiratory specimens with a *Mycobacterium tuberculosis* complex positive culture and a negative smear were selected retrospectively, among the left-overs of the Mycobacteria Laboratory of the University hospital, Lyon, France. Specimens were stored at -20°C before testing by Xpert MTB/RIF Ultra. For each sample, growth delay and the date of anti-TB treatment initiation were recorded.
Results: Among the 46 specimens, there were 29 sputum, 8 aspirates, 6 broncho-alveolar lavages and 3 stomach tubes. Thirty-two were collected before initiation of the anti-TB treatment. The results of Xpert MTB/RIF Ultra were collected in table below:

Total (collected before antibiotics) samples							
Growth delay MGIT Bactec 960 (Days)	Xpert MTB/RIF Ultra results						
	Positive					Negative	
	High	Medium	Low	Very low	Trace		
7-13	1 (1)	6 (4)	0	2 (2)	1 (1)	0	10 (8)
14-20	0	6 (1)	1	4 (3)	4 (4)	3 (3)	18 (11)
21-27	0	2	3 (2)	0	5 (4)	2 (2)	12 (8)
> 28J	0	0	1 (1)	1 (1)	2 (2)	1 (1)	5 (5)
	1 (1)	14 (5)	5 (3)	7 (6)	12 (11)	6 (6)	**45 (32)***

*one specimen gave an "error" result

The overall sensitivity was 87% (95% CI, 73.2, 94.9). Restrained to samples collected before treatment initiation, the sensitivity was 81% (95% CI, 63.6, 92.8). Xpert MTB/RIF Ultra gave a result for Rifampicin susceptibility status for 62% of specimens only, since 12 samples were paucibacillary ("trace" category). When available, the Rifampicin susceptibility status was fully concordant with the antibiogram.
Conclusions: The new test Xpert MTB/RIF Ultra improves pulmonary TB diagnosis, notably for paucibacillary smear-negative sputum, with a sensitivity of 81% compared to the culture.

P 78

Sensitivity comparaison of two real-time PCR kits for the in vitro *Mycobacterium tuberculosis* complexe diagnosis

Elisabeth Hodille[1], Alexia Barbry[2], Charlotte Genestet[3], Isabelle Fredenucci[1], Jean-Philippe Rasigade[1], Gérard Lina[4], Oana Dumitrescu[4]
[1]***Microbiology Department, Hospices Civils de Lyon, Hôpital de la Croix Rousse, Lyon, France***
[2]***Centre Hospitalo-Universitaire de Lyon, France***
[3]***CIRI INSERM U1111, University of Lyon, France***
[4]***Hospices Civils de Lyon, Centre National de Référence des Staphylocoques, CIRI INSERM U1111, France***

Background: *Mycobacterium tuberculosis* (MTB) infection is a major public health concern. Bacteria culture is considered as the gold-standard method for MTB infection diagnosis and is required to isolate strain for drug-sensitivity testing. Nevertheless, the Centers for Disease Control and Prevention recommend using at least one molecular technique per patient for MTB detection. Actually, for smear-negative samples, nucleic acid amplification (NAA) tests allow a rapid confirmation of MTB infection diagnosis compared to culture. Herein, we performed a retrospective study to compare performance of two real-time PCR kits for *in vitro* MTB DNA detection: RealCycler® MTBC using SmartCycler® (Hain Lifescience®) vs Fluorotype® MTB using FluoroCycler® (Biocentric®).
Methods: Specimens were retrospectively selected from those taken from patients during routine care, and analyzed in the Mycobacteria Laboratory of the Lyon University Hospital, France. A total of 50 pulmonary and extra-pulmonary smear-negative samples with a MTB-positive culture (growth delay between 5 and 20 days) were selected. Specimens were treated by the modified Kubica's digestion-decontamination method; smear staining was performed using the acridine orange method; and MTB cultures were performed using mycobacteria growth indicator tubes (MGIT) and the BACTEC 960® instrument. Specimens were stored at -20°C before extracting by Magna Pure Compact® and testing by RealCycler® MTBC and FluoroType® MTB. The comparison of sensitivities was carried out by a Mc Nemar test.
Results: Among the selected samples, there were 26 expectorations, 9 bronchial aspirations, 7 bronchoalveolar fluids, 4 tissue biopsies, 3 lymph nodes and 1 gastric aspirate.
The sensitivities were 70% (95%CI [55.39; 82.14]) and 46% (95%CI [31.81; 60.68]) using FlurorType® MTB kit and for RealCycler® MTBC kit respectively. The sensitivity of FluoroType® MTB kit was significantly higher than RealCycler® MTBC kit (p≤0.01, McNemar test).
Conclusion: On smear-negative samples, performance of FluoroType® MTB kit is better than RealCycler® MTBC kit. However, the RealCycler® MTBC kit performance is acceptable for the category of paucibacillary specimens.

P 85

Systematic evidence of the performance of the phenotypic non-commercial assays for the detection of *Mycobacterium tuberculosis* resistance to antituberculosis drugs

Vladyslav Nikolayevskyy[1], Jim Werngren[2], Kadri Klaos[3], Yen Holicka[1], Anh Tran[1], Irina Kontsevaya[4]
[1]***Public Health England, London, United Kingdom***
[2]***Unit for Laboratory Surveillance of Bacterial Pathogens, Department of Microbiology, Public Health Agency of Sweden, Solna, Sweden***
[3]***Tartu University Hospital, Estonia***
[4]***Institute of Organic Chemistry and Biochemistry, Czech Republic***

In 2011, several rapid non-commercial culture-based methods and assays were recommended by WHO for direct and indirect drug susceptibility testing (DST) of Mycobacterium tuberculosis (MTB) isolates. Through systematic reviewing of peer-reviewed publications published by June 2017, we aimed to summarise evidence on the performance of the non-commercial culture-based methods for detection of resistance to anti-TB drugs including microscopic observation of drug susceptibility (MODS), thin-layer agar (TLA) and colorimetric redox-indicator (CRI) assays.
Data derived from 43 publications satisfying selection criteria was in agreement with those published by WHO in 2011 confirming high sensitivity and specificity of MODS and CRI for direct and indirect detection of resistance to rifampicin (RIF) and isoniazid (INH). There were no significant differences between resazurin- and tetrazolium-based CRIs suggesting that performance of the assay does not depend on the colour indicator used. Pooled sensitivity and specificity values for indirect DST for ethambutol (EMB) using CRI assays was 94.0% and 82.0%, respectively, suggesting CRIs could be used to rule out resistance to EMB. Performance of CRIs for other drugs including pyrazinamide, fluoroquinolones and injectables,

and performance of TLA for direct and indirect DST varied more substantially across the reports reviewed in the current document.
The majority of reports included in the study were conducted in high TB, TB/HIV, and/or TB and multi-drug resistant (MDR) TB as well as resource-constrained settings. Excellent performance of MODS and CRI assays, respectively, for direct and indirect detection of resistance to key drugs confirmed a notion of the utility of non-commercial assays for a rapid and accurate detection of MDRTB in settings where the use of commercial WHO-endorsed culture-based assays could be limited due to a variety of reasons including lack of trained personnel and laboratory facilities.
Key factors currently affecting use of non-commercial culture-based assays for detection of resistance to anti-TB drugs identified in this study include safety considerations (especially for CRIs), cost-effectiveness as well as lack of standardization in drug concentrations (MODS, TLA) and breakpoint/cutoff values for CRI assays.

P 99

Phenotypic and genotypic characterization of resistance to bedaquiline and delamanid in *M. tuberculosis* clinical strains

Simone Battaglia[1], Emanuele Borroni[1], Andrea Maurizio Cabibbe[1], Matteo Chiacchiaretta[1], Maria Rosaria De Filippo[2], Daniela Maria Cirillo[1], CRyPTIC Consortium
[1]Emerging Bacterial Pathogens Unit, Division of Immunology, Transplantation and Infectious Diseases, IRCCS San Raffaele Scientific Institute, Milan, Italy
[2]London School of Hygiene and Tropical, London, United Kingdom

The increasing incidence of multi- and extensively- drug resistant tuberculosis (M/XDR-TB) represents a serious public health problem. Among new antituberculars for TB treatment, bedaquiline (BDQ) and delamanid (DLM) are the two most promising drugs. The molecular basis of resistance to these drugs is only partially understood. Mutations responsible for resistance to BDQ are found in *atpE*, which encodes for the ATP synthase c-F0 subunit and in *Rv0678*, a regulator which controls expression of MmpS5-MmpL5 efflux pump. Recently, it was demostrated that also mutations in *pepQ* gene could be involved in BDQ-R phenotype. Mutations leading resistance to DLM were found in *Rv3547* and in genes involved in F420 biosyntesis pathway. Previous studies demostrated that BDQ and DLM resistance may also occur in M/XDR-TB isolates without exposure to the drug. Therefore, knowledge of the molecular mechanisms leading to BDQ and DLM resistance is fundamental to address promptly the correct therapy. A total of 787 MTBC strains representing sixteen different lineages and all known drug susceptibility profiles were included in the study: minimal inhibitory concentrations (MICs) of BDQ and DLM were determined by using novel microtiter plates (UKMYC5, Thermo Fisher). MIC values of BDQ-R and DLM-R strains were confirmed by Bactec 960 MGIT system (BD) and resazurin microtiter assay (REMA) as reference methods. Genomic DNA was extracted by CTAB method for Whole Genome Sequencing (WGS) analysis. Paired-end libraries were prepared using Nextera XT DNA Sample Preparation kit and sequenced on NextSeq 500 platform (Illumina Inc). Samples showing a coverage of ≥ 30X were considered for SNPs calling performed by PhyResSE web-tool and the MTbseq pipeline. MIC values obtained by a microtiter plates revealed 7 BDQ-R and 4 DLM-R, confirmed by reference methods. WGS analysis on these strains revealed the presence of 7 non-synonymous mutations in the genomic loci investigated. Of the 7 BDQ-R strains only 5 harboured mutations in the candidate gene *Rv0678* (Arg38fs, 2 Ile67fs, Leu136Pro, Gly121Arg). The analysis of the 4 DLM-R strains genomes revealed the presence of 3 mutations in *ddn* gene (Trp20STOP, Leu90fs, Pro5fs) in 3 strains and the absence of mutations in the last DLM-R isolate. The analysis of susceptible strains revealed the presence of others non-synonymous mutations which do not correlate to drug resistance. The results of this genotypic-phenotypic correlation allowed to identify and discriminate SNPs correlated to DR phenotype from SNPs without any DR correlation. The final goal is to obtain an encyclopaedia of characterized mutations to identify DLM and BDQ resistant strains by WGS analysis. Finally, it is worth nothing that the population of strains were isolated from patients never exposed to DLM or BDQ, confirming that mutations inducing resistance phenotype are pre-existing in MTBC circulating strains.

P 100

Comparison of Decomics Decontamination Kit with Kubica Method for Recovery of *Mycobacterium tuberculosis* from Sputum

Meltem Ozden[1], Zeynep Saribas[1], Alpaslan Alp[1], Mutlu Hayran[2], Yakut Akyon[1]
[1]Faculty of Medicine, Department of Medical Microbiology, Hacettepe University, Ankara, Turkey
[2]Cancer Institute, Hacettepe University, Ankara, Turkey

The aim of this study, was to compare Decomics decontamination kit with the Kubica (NaOH and N-acetyl-L-cysteine (NALC)) decontamination method in sputum samples that had been spiked with *Mycobacterium tuberculosis*. Decomics is a decontamination and concentration kit that contains decontamination solution, absorbent beads and neutralization solution. It does not require centrifugation.
Serial dilutions *M. tuberculosis* H37Ra strain (10^8–10^1) were prepared in three ml of sputum. Two series of spiked bacterial suspensions were prepared. One of the series was used for decontamination with Decomics decontamination kit and the other for Kubica method. Each group was repeated for 37 times. After decontamination, samples were stained with Kinyoun staining; inoculated into Löwenstein-Jensen (LJ) and Middlebrook 7H10 media. Real-time PCR was performed for five repeats. In the acid-fast bacillus (AFB) staining of the samples, both methods detected AFB in concentrations between 10^8-10^4. In cultures, LJ detected 97%, 97%, 94%, 91%, 37% of 37 cultures decontaminated with Decomics; while 89%, 89%, 91%, 91% and 64% of them were detected with Kubica method, in 10^8, 10^7, 10^6, 10^5, 10^4 bacterial concentrations respectively. In Middlebrook 7H10 agar, colonies were detected in 97%, 100%, 94%, 97%, 54% and of the 37 repeats decontaminated with Decomics, and 89%, 94%, 89%, 91%, 78% of those decontaminated with Kubica method, in 10^8, 10^7, 10^6, 10^5, 10^4 bacterial concentrations respectively. No colonies were seen in lower concentrations decontaminated with either of the methods. Of the 37 repeats five of them were tested with real-time PCR. Both methods detected bacilli in concentrations of 10^8-10^6. Kubica method detected bacilli in 5, 4, 1 of the sputum repeats in concentrations of 10^5, 10^4 and 10^3 respectively. Decomics detected 3 repeats in the concentration of 10^5 and none in concentrations of 10^4 or 10^3.
According to our data it can be said that, Decomics efficiently decontaminates sputum and bacilli can be detected in the concentrations of approximately 10^8-10^5. Usage of Decomics decontamination kit can be helpful where tuberculosis is endemic and in areas where the laboratory facilities are inadequate since Decomics kit does not require centrifugation.

P 106

Line Probe assay (LPA) to identify Non-tuberculous Mycobacteria (NTM) isolates in a Southern Brazil reference hospital

Angelica C.A. Silva[1], Vinicius R. Belga[1], Lucas Menon[1], Renata H.C. Pocente[1], Erica Chimara[2], Valdes Bollela[1]
[1]School of Medicine of Ribeirão Preto, University of São Paulo, Brazil
[2]Instituto Adolfo Lutz Central, Brazil

Introduction: Non-tuberculous mycobacteria (NTM) are increasing in frequency around the globe. Potentially pathogenic NTM lead to various forms of disease, mainly in the of patients with pre-existing pulmonary diseases. The confirmatory diagnosis of NTM disease is complex and must follow a series of clinical, imaging and microbiological criteria.
Aim: to describe NTM detected among patients referred to tertiary hospital and to evaluate the role of a line probe assay to define the species of these Mycobacteria.
Methods: all the patients who had at least one NTM cultivated in MGIT from pulmonary and blood specimens, from Jan-2015 to Dec-2016, were included in this analysis. NTM species identification were made by the State Reference Lab (PRA-hsp65) and a sub-set tested with a line probe assay (Genotype *Mycobacterium*-CM, Hain-Lifescience-GmbH®) for 13 different NTM species, at Clinics Hospital of Ribeirão Preto Medical School (HCRP).
Results: In 2015, 124 NTM isolates were identified in the Central Lab (Adolfo Lutz Institute), 119 from pulmonary samples and five from blood samples. In 2016, 144 NTM isolates were identified in the Central Lab., 142 pulmonary and two blood specimens. All the seven isolates from blood sample were *M. avium*. From the other 261 isolates from pulmonary specimens, *M. intracellulare* were the most frequent with 136 confirmed (52,1%), *M. abscessus* with 30 (12,2%), and *M. avium* with 29 isolates (11,1%). After performing Genotype-CM in a total of 61 isolates, the concordance with the reference Lab result were present in 86.9% (53).
Conclusions: M. intracellulare is the most frequent NTM species detected in this region (Southern Brazil) among patients with pulmonary NTM disease and *M. avium* is still the most among NTM detected in HIV -patients. Genotype-CM CM is a reliable and fast tool to detect NTM species which may impact in the treatment

decision making test as the final results can be available in less than three days, after detection in MGIT culture.

Keywords: Non-tuberculous mycobacteria. Epidemiology. Clinical Laboratory Techniques. Molecular biology.

P 122

Genotyping of *Mycobacterium tuberculosis* by conventional and WGS based methods

Marjo Haanperä[1], Pieter Smit[2], Silja Mentula[1], Hanna Soini[1]
[1]***Department of Health Security, National Institute for Health and Welfare (THL), Helsinki, Finland***
[2]***Department of Infectious Diseases, Public Health Institute (GGD Amsterdam), Amsterdam, The Netherlands***

Genotyping of *M. tuberculosis* (TB) is rapidly shifting from spoligotyping and MIRU-VNTR typing to NGS-based analysis. We compared the results of whole-genome sequencing (WGS) and conventional genotyping for Finnish TB isolates. TB isolates from all culture-positive cases obtained in Finland in 2014, comprising 213 isolates from 211 patients, were analyzed by spoligotyping, MIRU-VNTR typing (24 loci), and WGS. Isolates with identical spoligo and MIRU results were considered to form a conventional genotyping cluster. For WGS, DNA libraries were generated using the Nextera XT kit and the libraries were sequenced with the MiSeq system (Illumina, San Diego, USA). Clustering analysis of the fastQ files was performed using Ridom SeqSphere+ (Ridom GmbH, Germany) cgMLST v2 (2891 targets) with genomes assembled by Burrows-Wheeler Aligner (bwa). Isolates with allelic distance ≤12 were assigned to a certain cluster type (CT) by SeqSphere+ and isolates with the same CT were considered to form a cgMLST cluster. In addition, SNP analysis using the GATK tools (Broad institute) was performed and clusters based on distance of ≤12 and ≤1 SNPs were formed.
The two paired isolates had the same results with all the methods except for SNP analysis by which 3 and 10 SNPs were found. Out of the 211 single isolates, 17 clusters comprising 41 isolates (19 %) were found by conventional genotyping. Of these, 17 (41,4 %) were clustered similarly to seven clusters also by SeqSphere CT analysis. Furthermore, four strains not clustered by conventional genotyping clustered with SeqSphere CT analysis resulting in eight clusters with 21 (10 %) clustered isolates. By ≤12 SNPs clustering cut-off, 8 clusters with 18 isolates were detected. One of these isolates was not clustered by SeqSphere+ CT analysis although the allelic distance among the isolates of this three-isolate SNP cluster was 2-10. With the 1 SNP cut-off, three clusters with seven strains were found and these were similarly clustered also by SeqSphere+ and conventional genotyping analysis.
In conclusion, WGS analysis less clusters and more precise genotyping information compared to conventional methods, as highlighted by the results of the paired isolates and the strains with partly ambiguous or inconsistent conventional genotyping results clustered by WGS. In addition, due to the large number of genotyping targets in WGS, difference or even absence of a result for one or a few targets may not change the result dramatically. SNP analysis was the most discriminatory method.

P 123

Comparison of GeneXpert Mtb / RIF test results with culture and microscopy in pulmonary and extrapulmonary samples

Derya Altun[1], Ahmet Arslantürk[2], Hulya Simsek[3], Alper Sarıbaş[1], Nilay Uçarman[1]
[1]***National Tuberculosis Reference Laboratory, Ankara, Turkey***
[2]***Public Health General Directorate, Ministry of Health of Turkey, Ankara, Turkey***
[3]***Ministry of Health, General Directorate of Public Health, National Tuberculosis Reference Laboratory, Ankara, Turkey***

Objective: The purpose of this study was to test the efficiency and reliability of the GeneXpert system for the detection of M. tuberculosis bacteria in pulmonary and extrapulmonary specimens and to compare it with culture, microscopy and drug susceptibility test results.
Materials and Methods: The study included culture or ARB microscopy negative 21 samples with culture or ARB microscopy positive 47 samples isolated in the National Tuberculosis Reference Laboratory in 2017 and culture or ARB microscopy positive 21 samples isolated from Süreyyapaşa Chest Diseases and Thoracic Surgery Training and Research Hospital. All samples included in the study tested with GeneXpert MTB / RIF test, ARB microscopy (Erlich-Ziehl Neelsen method) and culture (Löwenstein Jensen and MGIT automated culture system).
Findings: Of the samples included in the study, 36 (40.5%) were extrapulmonary and 53 (59.5%) were pulmonary samples.
Positive results were obtained with the GeneXpert MTB / RIF test on culture-positive and ARB microscopy-positive on 24 of 29 samples. Positive

results were obtained with the GeneXpert MTB / RIF test on culture-positive and ARB microscopy-negative on 19 of 32 samples.
Negative results were obtained with GeneXpert MTB / RIF test in culture-positive 18 of 61 samples and 9 of these samples were TDM, 9 were Mtbc. They are determined by immunochromatographic test.
Negative results were obtained with both the GeneXpert MTB / RIF test and ARB microscopy in culture-negative 28 samples and it was understood that they were negative specimens.
One sample negative for culture and ARB microscopy was found positive with the GeneXpert MTB / RIF test, and when retrospectively examined it was found that it is belong to a patient under treatment.
Forty-three (89.5%) of the 48 samples identified positive by GeneXpert MTB / RIF test were cultured and 4 (8%) were found to have RIF resistance.
Result: GeneXpert Mtb-Rif test has an important role in early diagnosis of tuberculosis and early detection of MDR-TB in pulmonary and extrapulmonary samples. Early detection of rifampin resistance contributes to the regulation of the treatment of patients. GeneXpert MTB / RIF test results should always be verified with culture and IDT results.
When positive results are obtained with the ARB microscopy or the GeneXpert MTB / RIF test, the patient should be considered to be under treatment while the culture is negative.

P 124

Identification of the Apa protein secreted by *Mycobacterium avium* subsp. paratuberculosis as a potential fecal biomarker for the immunodiagnosis of Johne's disease in cattle

Giliane Silva Souza[1], Ana Barbara Rodriguez[1], Maria Isabel Romano[2], David Gitirana Rocha[1], Wilmar Dias Silva[4], Elena Lasunskaia[1]
[1]Universidade Estadual Do Norte Fluminense, Campos/Rio de Janeiro, Brazil
[2]INTA, Biotechnology Institute, Buenos Aires, Argentina
[4]Instituto Butantan, Universidade Estadual Do Norte Fluminense, Campos, Brazil

Paratuberculosis (PTB) or Jonhe's disease is a chronic intestinal infection of ruminants, caused by *Mycobacterium avium* subsp. *paratuberculosis* (Map). Isolation of Map cultures from the feces of suspected animals provides definitive diagnosis of the infection. However, the bacteriologic method is time-consuming, as Map is one of the slowest growing mycobacterial species that needs months for growth in the specific mycobactin-supplemented medium. The low-level shedding of Map with feces starts at the initial stages of infection and increases along the disease suggesting that antigens secreted by mycobacteria could be excreted with feces. Immunodiagnostic approach, based on detection of microbial antigens in the fecal samples (fecal ELISA or rapid immunochromatographic assay), was successfully employed for the non-invasive diagnosis of intestinal infections in humans, such as infections caused by *Helicobacter pylori*, *Giardia lamblia* or *Cryptosporidium parvum*. Antigens that could serve as fecal biomarkers for the Jonhe's disease are currently unknown. In previously study (Souza G. et al., 2011), we demonstrated that the alanine and proline-rich antigen (Apa), a major secretory antigen of Map, could be detected in intestine of cows with PTB by specific monoclonal antibody (mAb). In this study, we verified whether this protein can be found in consistently detectable levels in feces of cattle with PTB. The feces, obtained from 1) cows with Johne's disease confirmed by laboratory tests; 2) cows suspected in PTB based on seropositivity for anti-Map, and 3) PTB-free control cows, were immunoprecipitated by the anti-Apa mAb and analysed by immunoblot. The Apa glycoprotein was detected as a 60/70 kDa doublet band in all samples obtained from the animals with laboratory-confirmed disease and in the substantial proportion of seropositive asymptomatic animals, but not in the control samples. Additionally, the antigen was detected in feces of animals with Johne's disease by novel kit developed for anti-Apa sandwich ELISA. This study identifies Apa as a potential fecal biomarker of Johne's disease in cattle that could serve for immunodiagnosis.
Financial support: FAPERJ, CNPq

P 126

Comparison with FluoroType MTBDR Assay and Culture for the Detection of *Mycobacterium tuberculosis* and Resistance to Rifampicin and Isoniazid, And Concordance between FluoroType MTBDR Assay and Phenotypic Drug Susceptibility Testing

Hulya Simsek[1], Derya Altun[1], Ahmet Arslanturk[1], Alper Sarıbaş[1], Nilay Uçarman[1]
[1]Ministry of Health, General Directorate of Public Health, National Tuberculosis Reference Laboratory, Ankara, Turkey

Aim: The elimination of tuberculosis has been more challenging since the diagnosis and treatment of patients with multidrug-resistant *M.*

tuberculosis (MDR-TB) are difficult. We need rapid and accurate diagnostic techniques for preventing transmission of MDR-TB in society. We aimed to evaluate FluoroType MTBDR, recently a new molecular diagnostic test, in comparison to mycobacterial culture method and phenotypic drug susceptibility testing (DST) of *M. tuberculosis* complex (MTBC).**Method:** 48 different pulmonary and extrapulmonary samples were selected in the last six months. After processed, all samples were stained and cultured using MGIT 960 and LJ slants. At same time, all samples were tested by FluoroType MTBDR (Hain Lifescience) according to manufacturer's instructions. All cultures were differentiated from MTBC and NTM. DST for MTBC were done by MGIT 960.
Results: Of 24 pulmonary samples, 18 (13 positive and 5 negative for AFB) were culture positive, and 6 (1 positive and 5 negative for AFB) were culture negative. Of 24 extrapulmonary samples, 19 (4 positive and 15 negative for AFB) were culture positive and 5 (1 positive and 4 negative for AFB) were culture negative. Nine samples identified as NTM were excluded. Culture was evaluated as gold standard. Sensitivity and specificity of FluoroType MTBDR were 77% and 100% respectively for pulmonary samples, while positive predictive value (PPV) and negative predictive value (NPV) were 100% and 63% respectively. For extrapulmonary samples, sensitivity and specificity of FluoroType MTBDR were 69% and 100% respectively, while PPV and NPV were 100% and 50% respectively. Of 13 culture-positive pulmonary samples, 9 rifampicin (RIF) and 6 isoniazid (INH) susceptibility by FluoroType MTBDR were concordant with phenotypic DST, 5 isolates were negative and 4 isolates were found positive, but their resistance could not be detected by FluoroType MTBDR.
Conclusion: This study has shown 100% specificity of the FluoroType MTBDR for pulmonary and extrapulmonary samples. But it is considered that sensitivity and NPV of both samples are low. It is concluded that it requires a new study with the number of more samples for detecting MTBC and its INH and RIF resistance, and to validate the test.
Key words: FluoroType MTBDR Assay, multidrug-resistant *Mycobacterium tuberculosis*, diagnosis

P 127

WGS as a tol for diagnosing drug- and multidrug-resistant tuberculosis in Mexico

Carlos Madrazo-Moya[1], Betzaida Cuevas-Cordoba[2], Jose Zarrabal[3], Iñaki Comas[4], Aurora Parissi-Crivelli[3], Ana JImenez-Ruano[1], Nancy Seraphine[5], Vanessa Gonzalez[2], Michael Lauzardo[5], Roberto Zenteno-Cuevas[1]
[1]Public Health Institute and Health Sciences Institute, Universidad Veracruzana, Mexico
[2]Pharmacogenomics Laboratory, The National Institute of Genomic Medicine, Mexico City, Mexico
[3]Laboratorio Estatal de Salud Pública, Secretaria de Salud, Veracruz, Mexico
[4]Unidad de Genómica de la Tuberculosis, Instituto de Biomedicina de Valencia, Consejo Superior de Investigaciones Científicas, Valencia, Spain
[5]Division of Infectious Diseases and Global Medicine, College of Medicine, University of Florida, Gainesville, Florida, United States

Introduction: In Mexico 2.5% of the 23,000 new cases of tuberculosis (TB) annually diagnosed have resistance against one drug, also Mexico have a prevalent population of 600 MDR-TB cases and increasing numbers of XDR-TB cases. These figures evidence the need for alternative procedures that promotes a fast and reliable diagnostic of drug resistance. For these reason in this study we estimate the worth of whole genome sequencing (WGS) as a diagnostic tool for tuberculosis drug-resistant in Mexico.
Methods: Using WGS and an analysis pipeline established by our team, we identify single nucleotide polymorphisms (SNP) associated with drug resistance and strain lineage assignation for 75 clinical *mycobacterium* tuberculosis isolates collected in Mexico. Phenotypic resistance against first line drugs had previously been established using culture (MGIT system). In addition, we identified clonal complexes using and correlated with epidemiological data of the individuals.
Results: Of the individuals bearing the isolates 46 (61%) were male, the mean of age was of 45.3 and according to the drug-resistance profile, 21 (28%) were mono-resistant, 11 (15%) poli-resistant and 43 (57%) MDR. More than 50 different SNPs in genes related with drug resistance were found in the 75 genomes analyzed. The most frequent mutation associated with resistance against isoniazid was katG315Ser/Thr (31/58), to rifampicin rpoB450(531)Ser/Leu (31/46), ethambutol embB306Met/Val (9/23) and pyrazinamide pncA120Leu/Pro (13/29). One isolate had the polymorphisms that placed it as pre-XDR and one as XDR-TB. Sensitivity and specificity for drug against isoniazid was 85% and 100%, toward rifampicin 91% and 93%, to ethambutol 70% and 88%, to pyrazinamide 72% and 94%, and for MDR-TB 86% and 100%. We classified the 75 genomes into eight lineages, the most frequent being H and X with 21 isolates each. Six clonal complexes (CC) were found; one included twelve MDR-TB isolates of X lineage and includes the pre- and XDR-TB isolate, all

isolates in this cluster were located in two major cities, Jalapa and Veracruz-Puerto.
Conclusions: Our results confirm that WGS could be a suitable tool for the diagnostic of rifampicina, isoniazid and MDR-TB in Mexico. The possibility to identify isolates with pre-XDR and XDR condition, and lineage classification were results that give to the WGS an additional value. Therefore, this procedure could be a powerful tool to support the control of drug-resistant tuberculosis in Mexico.

P 141

Comparison between xpert MTB/RIF ultra and nested PCR for identification of *M. Tuberculosis* complex in formalin fixed paraffin embedded specimens

Anna Camaggi[1], Claudia Veggiani[2]
[1]Laboratory of Microbiology and Virology, Maggiore Della Carità University Hospital, Novara, Italy
[2]Pathology Unit of University Hospital, Maggiore Della Carità, Novara, Italy

Background: The rapid and accurate diagnosis of *M. tuberculosis* complex (MTB) infection is essential for the optimal treatment of tuberculosis (TB). WHO recommends the use of rapid and sensitive methods providing diagnosis and information on drug resistance.
In suspicious of tuberculosis, fresh specimens are sent in microbiology laboratory and submitted to molecular assay, acid-fast microscopy and culture (both solid and liquid media).
Conversely, when a tuberculosis infection is not clinically suspected, tissues are usually sent in Pathology Unit where they are formalin-fixed and paraffin-embedded (FFPE) for diagnostic purposes. In case of histologic suspicious of tuberculosis infection, they can be submitted to DNA extraction and nestedPCR (nPCR) targeting IS6110 for MTBC, without additional information on drug resistance.
The aim of this study was to compare two molecular systems: Xpert MTB/RIF Ultra (Ultra) (Cepheid, Sunnyvale, USA), usually used on fresh tissue to nPCR on FFPE tissue.
Materials and Methods: Thirty FFPE samples from different human district were submitted to molecular analysis after deparaffination. All samples were selected by our TB cases archive: 24 positive and 6 negative.
In Pathology Unit the samples were processed with nPCR using J and K, outer primer and IS1and IS2, inner primer to amplify a 121 bp fragment of IS6110 of *M. tuberculosis* complex.
In microbiology laboratory, Ultra was used. Ultra use two different multi-copy amplification targets (IS*6110* and IS*1081*) and melting temperature-based analysis.
Results: In 28 cases the results of both methods were the same: 22 cases were MTB positive (1 MTB detected high, 4 MTB detected low, 7 MTB detected very low and 10 MTB trace detected) and 6 negative. Conversely, in two cases the results were discordant: Ultra was "MTB detected trace" and nPCR was negative. Ultra information on rifampicin resistance for TB positive results were: 12 rifampicin resistance not detected and 12 unknown rifampicin resistance ("MTB trace detected" result provides no information on rifampicin resistance). Culture archive results confirm Ultra results.
The results by nPCR were obtained in one day and half and in less than five hours using Ultra.
Conclusion: Ultra is faster, reliable, very sensitive also on FFPE tissue and supply the information to the resistance to rifampicin. Sometimes, on FFPE tissue is more difficult reliable rifampicin resistence for low DNA presents in samples but certainly the Xpert Ultra is faster than nPCR.

P 147

Xpert Ultra – improving detection at what cost?

Shaheed Vally Omar[1], Farzana Ismail[2], Elliot Elawani Marubini[1], Lavania Joseph[1], Nazir Ismail[2]
[1]Centre for Tuberculosis (WHO TB SRL Network), National Institute for Communicable Diseases, National Health Laboratory Service, Johannesburg, South Africa
[2]Department of Medical Microbiology, University of Pretoria, South Africa

The World Health Organization identified drug resistant tuberculosis as a major public health concern which threatens the progress made in the control of this disease. The introduction of the Xpert MTB/RIF assay has revolutionized the diagnostic landscape for the rapid detection of tuberculosis and drug resistance and key to ENDing TB. Since the South African implementation of the Xpert as the first-line diagnostic in 2010, several versions of the assay have been released. In late-2017, the Xpert Ultra cartridge was introduced based on its improved sensitivity for detecting TB and similar performance for detection of rifampicin resistance compared with the G4 cartridge it now replaces. The Ultra differs significantly from the G4 amongst them a change in the genetic targets and the use of melt-curve analysis for rifampicin resistance determination. In this study, we evaluated the ability of the Ultra to detect the

circulating drug resistance associated rifampicin mutations in South Africa.
This Study was performed at Centre for Tuberculosis at South Africa's National Institute for Communicable Diseases. The Whole Genome Sequencing DNA repository was used and the different drug-resistance conferring *rpoB* mutations within and outside the RRDR was selected. Forty-Eight (48) unique strains were identified representing 27 different *rpoB* mutations within the RRDR and 1 outside this region. A maximum of 3 unique clinical strains shared the same mutation. Strains were sub-cultured and processed on the GXP Ultra. Results were captured and melt-curves analysed. Discordant isolates were repeated on the GXP Ultra as well as the GXP G4 cartridge and the repeat results reported.
The GXP Ultra cartridge detected 24/27(88.9%) *rpoB* mutants within the RRDR region. Of the 3 mutants not detected by the Ultra cartridge even on repeat testing, 2 were missed completely and reported as WT while 1 was reported as indeterminate. The GXP G4 cartridge positively detected all 3 mutants. The single mutation found outside the RRDR was not detected by both assays as expected.
In conclusion, despite the improvement of the Xpert Ultra to increase the sensitivity of detecting the presence of *Mycobacterium tuberculosis* in suspected TB patients, the cost of this may result in missing drug-resistance, not previously missed by the Xpert G4. This has significant implications, as patients may be placed on incorrect treatment regimens resulting in further spread of drug-resistant TB and poor treatment outcomes, despite the disease being detected early.

P 153

Lack of association of novel mutation aftB D397G with Ethambutol resistance in clinical isolates of *Mycobacterium tuberculosis* reveals the necessity of genotyping

Astha Giri[1], Shraddha Gupta[1], Andrea M. Cabibbe[2], Kamal Shrivastava[1], Anshika Narang[1], Alberto Trovato[2], Simone Battaglia[2], Naresh Sharma[1], Daniela Maria Cirillo[2], Mandira Varma-Basil[1]
[1]Dept of Microbiology, Vallabhbhai Patel Chest Institute, University of Delhi, India
[2]Emerging Bacterial Pathogens Unit, Division of Immunology, Transplantation and Infectious Diseases, IRCCS San Raffaele Scientific Institute, Milan, Italy

The present molecular diagnostic assays for ethambutol (EMB) resistance only include polymorphisms at *embB306* which accounts for 40-60% of EMB resistance found globally. In order to find additional genotypic indicators for EMB resistance, polymorphisms were studied in arabinofuranosyltransferase encoding genes *aftA* (Rv3792), *aftB* (Rv3805) and *aftC* (Rv2673) in addition to the *embCAB* operon in 28 EMB resistant and 30 EMB susceptible isolates from India using Sanger sequencing and Illumina whole genome sequencing. The results were further correlated with the minimum inhibitory concentration (MIC) and the effect of the non-synonymous polymorphism D397G in *aftB* on MIC was analysed by an over-expression approach.
The polymorphism D397G in *aftB* was found in 7/28 (25%) EMB resistant strains and 1/30 (3.3%) EMB sensitive isolates, while no significant mutations were observed in *aftA* or *aftC.*
Though all the isolates with the *aftB* D397G mutation also carried an *embB306* mutation (6/7; 85.7%) or an *embB402* mutation (1/7; 14.3%), the association of the D397G polymorphism with EMB resistance was found to be statistically significant by SNP analysis (p=0.0232, Fischer exact test). Interestingly, 5/7 (71.4%) of the isolates with the D397G mutation had high-level EMB resistance (MIC≥ 16ug/ml), 1/7 (14.3%) had low level resistance to EMB (MIC 4ug/ml), while MIC was not available for one isolate. Overexpression of the mutant *aftB* in H37Rv did not exhibit any change in the MIC. Whole genome sequencing of a panel of isolates confirmed the results of sequencing and also revealed that the mutation D397G at *aftB* was associated with only the Beijing genotype in a clonally diverse population of *M. tuberculosis* isolates.
Hence, though *aftB* D397G mutation was found to be significantly more in high level EMB resistant *M. tuberculosis* isolates than EMB susceptible isolates, overexpression analysis and genotyping by whole genome sequencing revealed that the mutation was not associated with EMB resistance and was instead a phylogenetic marker for Beijing isolates. The study also highlights the use of whole genome sequencing to identify the role of novel mutations in *M. tuberculosis* isolates in a high burden country.

P 155

Whole-genome sequencing characterization of *Mycobacterium tuberculosis* drug-resistance profiles at a referral centre in Rome: implications for diagnosis and diseases control

Angela Cannas[1], Ornella Butera[1], Chiara De Giuli[1], Antonio Mazzarelli[1], Carolina Venditti[1], Gina Gualano[1], Fabrizio Palmieri[1], Enrico

Girardi[1], Antonino Di Caro[1]
[1]National Institute for Infectious Diseases IRCCS L. Spallanzani, Rome, Italy

Introduction: Control of tuberculosis (TB) has been effective especially in low-incidence countries, nevertheless the gains are threatened by the increasing burden of multidrug-resistant (MDR) and extensively drug-resistant (XDR) disease.
The metropolitan area of Rome has experienced recently intense migration waves, resulting in higher rates of TB compared to Italy as a whole. A clear knowledge of the trends of drug-resistant TB in this area could contribute to a better management of the disease. The use of whole-genome sequencing (WGS) could help to identify M. tuberculosis mutations correlated with drug-resistance.
In this study we aimed to characterize, using WGS, the drug-resistance profiles of TB strains circulating in the great metropolitan area of Rome.
Methods: *M. tuberculosis* strains isolated from patients admitted at the National Institute for Infectious Diseases in Rome between 2011 and 2016 with TB, were subjected to DNA extraction (CTAB method) and to WGS by Illumina. Sequences were uploaded on PhyResSE 1.0 for identification of mutations correlated with drug-resistance.
Phenotypic drug-susceptibility testing for the first line drugs was performed on all strains, as part of the diagnostic routine.
Results: *M. tuberculosis* isolates were collected from 260 patients and sequenced. Of these, 193 (74%) were from foreign born (FB) patients. Mutations correlated to drug-resistance were found in 17% of the strains, and approximately half (54.5%) of these were MDR. Resistance profiles including single or multiple drugs were characterized and further compared to phenotypic results. Agreement of 94% was found on MDR strains, while second line drugs show more significant differences, although phenotypic DST was done on limited number of them.
Conclusions: This study, conducted in a TB referral hospital in Rome, provides information on drug-resistance related mutations found in strains circulating in the area. Discordant results were evaluated. A panel of single and multiple drug-resistances was found and further investigation is ongoing.

P 167

Europpean inter-laboratory comparison of variable number tandem repeat typing and whole genome sequencing

Richard Anthony[1], Participants of EQA 2017[2], Rana Jajou[1], Dick van Soolingen[3], Vladyslav Nikolayevskyy[4]
[1]RIVM National Institute for Public Health and the Environment, Bilthoven, The Netherlands
[2]Belgium, Bulgaria, Croatia, Czech Republic, Denmark, Finland, France, Germany, Ireland, Italy, Luxembourg, Norway, The Netherlands, United Kingdom, Slovenia, Sweden, Spain
[3]National Institute for Public Health and the Environment, Bilthoven; Department of Medical Microbiology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands
[4]Public Health England, London, United Kingdom

Sixteen European laboratories participated in the 2017 ERLTB-Net TB genotyping EQA round, of which four performed WGS only, four laboratories MIRU-VNTR only, and the remaining nine laboratories both MIRU-VNTR and WGS. Within this round, 10 DNA samples were sent to the laboratories for both 24-locus MIRU-VNTR analysis and WGS analysis. For the MIRU-VNTR analysis, overall patterns and common problems were similar to those reported for 2016 EQA round with most laboratories scoring well and a few laboratories experiencing systematic errors resulting in very low scores.
Samples with varying levels of difficulty were included in the EQA panel: DNA's 2 & 6 and DNA's 3 & 5 were duplicates derived from the same isolate; DNA 4 was a *M. canetti*, DNA 1 was epidemiologically linked to DNA 3 & 5; DNA 8 was an MDR-TB; and DNA's 7 & 9 were also epidemiologically linked. The intra laboratory reporting on the relation between isolates varied between laboratories but there was only one truly incorrect call which was a clerical error. In general, all laboratories correctly identified genetically identical isolates except for three laboratories that reported 1 – 36 SNPs between the duplicates. The genetic distance reported between isolate 1 and 9 (genetically closely related but not epidemiologically linked) ranged from 23 – 81 SNPs. All laboratories correctly identified DNA 4 as *M. canetti* and also all but one laboratory correctly reported DNA 8 as MDR-TB. Inter laboratory identification of clones was not assessed this year due to lack of defined reporting standards.
Variation in WGS results between laboratories can be caused by several factors/settings applied in the WGS analysis pipeline of respective laboratories. Despite this, the results reported by laboratories was highly comparable; the minor differences observed were attributable to interpretation and reporting errors. To analyse to what extent the sequence technique and subsequent data processing contributes to a

variation, laboratories were requested to share their raw sequence data (FastQ files) for the 10 DNA samples. These raw sequence data were analysed within one pipeline to eliminate the influence of the pipeline so observed variation can only be attributed to sequencing itself.
The apparent robustness of WGS analysis suggests cross border comparisons will be possible, EQA can contribute to identifying weakness in reporting, and that the WGS technique has the potential to be reliable for this application.

P 178

Genomic of MDR and XDR-TB in Kazakhstan by combination of high throughput methods

Bernice Klotoe[1], Emilyn Conceição[2], Sarah Kacimi[1], Sikhayeva Nurgul[3], Harrison Magdinier Gomes[4], Sarah Sengstake[5], Anna Schuitema[6], Arike Alenova[7], Elena Zholdybayeva[3], Richard Anthony[8], Guislaine Refrégier[1], Christophe Sola[1]
[1]Institute for Integrative Biology of the Cell (I2bc), CEA, CNRS, Univ. Paris-Sud, Université Paris-Saclay, France
[2]Instituto de Microbiologia Professor Paulo Góes, Universidade Federal Do Rio de Janeiro, Brazil; Institute for Integrative Biology of the Cell (I2bc), CEA, CNRS, Univ. Paris-Sud, Université Paris-Saclay, France
[3]National Centre for Biotechnology, Astana, Kazakhstan
[4]Institute for Integrative Biology of the Cell (I2bc), CEA, CNRS, Univ. Paris-Sud, Université Paris-Saclay, France; Laboratory of Molecular Biology Applied to Mycobacteria, FIOCRUZ, Rio de Janeiro, Brazil
[5]Unit of Mycobacteria, Institute of Tropical Medicine, Antwerp, Amsterdam; Royal Tropical Institute, Amsterdam, The Netherlands
[6]Royal Tropical Institute, Amsterdam, The Netherlands
[7]National Centre for Tuberculosis Problems, Almaty, Kazakhstan
[8]Royal Tropical Institute, Amsterdam; National Institute for Health and Environment, Bilthoven, The Netherlands

A convenience sample of 700 DNA extracted from *Mycobacterium tuberculosis* cultures isolates from as many as 630 Kazakhs tuberculosis patients, recruited within 12 out of 14 regions in Kazakhstan between 2010-2015 was independently studied by two high-throughput hybridization-based methods, TB-SPRINT (59-Plex, n=700), TB-SNPID (50-Plex, n=543). Almost 400 (n=391) clinical isolates DNA were typed by both methods. Two lineages only, L2 and L4, were detected. L2 (Beijing lineage) prevalence totalled almost 80% by the two methods. MDR-TB was present in 50% of all samples studied by either TB-SPRINT or TB-SNPID (n=273/543). When a dynamic landscape of *M. tuberculosis* genotypes and of drug-resistance evolution is drawn, from 2005 till today, an evidence of an increase of spread of a limited number of L2/Beijing clones is observed. A more precise identification of the L2 isolates (Beijing) was performed using analysis of the IS*6110* in the NTF region, showing that all L2 isolates were Modern Beijing isolates. Further partial VNTR results on L2 isolates on a set of 8 loci to study the prevalence of the previously described 94-32 epidemic clone confirms the prevalence of this clone and its SLV and DLV variants. A fine spatial genetic map analysis confirms the history of the MDR-TB outbreak in Kazakhstan which is made up of a limited number of L2 clones that underlines the ongoing MDR-TB and possibly XDR-TB transmission. Although the incidence of TB is decreasing in Kazakhstan, we demonstrate that the proportion of small number of highly resistant MDR-TB is increasing due to their efficient transmission. Current treatment protocols do not prevent the spread or cure these strains and identifiying them as well as identifiying and applying effective treatment in a timely manor will be needed to attain the ambitious goals for TB control.

P 183

Comparison between spoligotyping and Region of Difference PCR for identification of M. bovis and BCG strains in Turkey

Begum Kayar[1], Gulfer Yakici[2], Emel Yarar[2], Firat Karsli[2], Ali Uckayabasi[2], Manaf ElMatar[2], Tulin Gokmen[2], Togrul Nagiyev[2], Fatih Koksal[2]
[1]Tropical Disease Research and Application Center; Faculty of Health Science, Cukurova University, Adana, Turkey
[2]Medical Faculty Microbiology Dep., Cukurova University, Adana, Turkey

Background: BCG is difficult to differentiate from other strains of M. bovis and other members of the M. tuberculosis complex by conventional methods. The molecular methods that demonstrate the presence of RD regions, which also play an important role in the evolutionary process, are very important at rapid diagnosis. We aimed to make this distinction by performing multiplex PCR with 6 RD regions taking into account the evolutionary tree of Mycobacterium tuberculosis complex.

Objective: Molecular identification of M. bovis strains by spoligotyping and multiplex PCR of 6 regions of difference; RD9, RD12, RD13, RD14, RD4 , RD1.
Materials and methods: 100 strains of the M. tuberculosis complex, isolated during 2007-2017 in the Region Tuberculosis Laboratory in Adana, were used in this study; 60 strains of M. bovis, 20 strains of M. tuberculosis, and 20 strain of M. bovis BCG. These strains were already identified using biochemical tests. Genomic DNA was obtained by the mickle method. All strains were tested by multiplex PCR based on the presence (+) or absence (-) of six RD regions. Spoligotyping was performed to confirm sublineage.
Results: The molecular identification by multiplex PCR based on the presence or absence of RD regions shows that 60 strains were M. bovis, 20 strains belonged to the group M. tuberculosis, 20 strains identified as M. bovis BCG. The molecular identification of M. bovis by RD has a sensitivity and a specifity of 100%. Spoligotyping tests ongoing.
Conclusion: The Identification by the 6 RD allows accurate and rapid identification of M. bovis and BCG strains with low cost.

P 186

An in- house Duplex PCR assay used for identification of *Mycobacterium tuberculosis* as an abutment of MGIT TBc ID test

Chanchal Kumar[1], Kamal Shrivastava[1], Naresh Sharma[1], Jitender Yadav [1], Mandira Varma-Basil[1]
[1]Dept. of Microbiology, Vallabhbhai Patel Chest Institute, University of Delhi, India

Introduction: Routinely, identification of *M. tuberculosis* complex (MTBC) and non tuberculous mycobacteria (NTM) is achieved by their growth characteristics in culture and biochemical tests, which are labour-intensive, time-consuming and often inconclusive. The high costs of commercially available assays have restricted their use in most clinical laboratories, especially in high burden countries endemic for tuberculosis. In our attempt to develop a simple and cost effective assay to rapidly differentiate MTBC from NTM. We explored a duplex PCR assay and compared the assay with the MGIT TBc Identification Test (TBc ID) for identification of *Mycobacterium tuberculosis* complex.
Methodology: Culture positive BACTEC MGIT960 tubes were obtained from the Department of Microbiology, Vallabhbhai Patel Chest Institute, Delhi and subjected to Ziehl-Neelsen (ZN) staining and TBc Identification test (TBc ID) as per the manufacturer's recommendations. Randomly selected 126 cultures were further analysed by an in-house Duplex PCR assay targeting *hsp65* and *Rv1458c* genes. Amplification of only *hsp65* indicated the presence of NTM which were further tested by Hain's Genotype Mycobacterium CM/AS.
Results: The culture positive MGIT tubes (n=125) tested by TBc ID were categorised as TBc ID positive (n=10), TBc ID faint positive (n=42) and TBc ID negative (n=73). ZN staining of the cultures revealed that 10/10 (100%) TBc ID positive, 22/42 (52.5%) TBc ID faint and 44/73 (60.2%) TBc ID negative cultures were AFB smear positive. All the cultures were also evaluated for the presence of mycobacteria by Duplex PCR. As expected, none of the AFB smear negative cultures were positive by Duplex PCR. Of the MGIT cultures that were AFB positive, 10/10 (100%) TBc ID positive, 9/22 (41%) TBc ID faint and 5/44 (11.3%) TBc ID negative cultures were identified as *M. tuberculosis* complex (MTBC) by the duplex PCR assay. The remaining AFB smear positive cultures were identified as NTM. The NTM were further identified to species level by Genotype Mycobacterium CM/AS assay. Duplex PCR and TBc ID assay failed to identify MTBC in one culture which was later confirmed by Line probe assay.
Conclusions: The MGIT TBc ID assay can be misinterpreted or can miss the presence of MTBC and an adjunct assay may help improve the sensitivity.

P 192

Comparison of Newly Developed BD MAXtm MDR-TB panel and DNA sequencing method for molecular diagnosis and resistance of *Mycobacterium tuberculosis* complex strains

Gulfer Yakici[1], Begum Kayar[2], Emel Yarar[1], Firat Karsli[1], Ali Uckayabasi[1], Manaf Elmatar[1], Togrul Nagiyev[3], Fatih Koksal[3]
[1]Medical Microbiology Department, Faculty of Medicine, Cukurova University, Adana, Turkey
[2]Medical Faculty, Microbiology Dept; Tropical Disease Research and Application Center, Cukurova University, Adana, Turkey
[3]Tropical Disease Research and Application Center, Cukurova University, Adana, Turkey

Introduction: Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* complex (MTBC). It remains one of the major health problem because TB is the ninth leading cause of death worldwide. Therefore rapid and accurate molecular diagnosis of drug resistance can contribute to overcome this problem.

In this study, the objective was to compare accuracy and effectiveness of newly developed real time PCR system BD MAX™ MDR-TB panel and DNA sequencing of *rpoB, katG and inhA* genes among patients which were considered as MDR by using phenotypic DST.
Material and methods: A total of 100 MDR-TB strains were included in our study. Samples were randomly selected among isolates which phenotypic drug susceptibility tests were performed on BACTEC-MGIT 960 at the, Adana Regional Tuberculosis Laboratory.
For sequencing DNA was extracted from positive culture (MGIT) by using mechanical extraction method following step was PCR of the hsp65, *rpoB*, *katG* and *inhA* genes. On the BD Max system after addition of isolates into strips, it performs extraction and real-time PCR automatically. DNA extracts were analyzed for genotypic resistance by MDR-TB panel on the BD Max system and target sequencing by using ABI prism 310 Genetic Analyzer platform.
Results: We analyzed 100 MDR-TB isolates to compare accuracy and power of the BD MAX™ MDR-TB panel to diagnose MTBC and detect rifampicin and isoniazid resistance and DNA sequencing. According to results of analyzed 100 MDR isolate, phenotypic and genotypic drug resistance testing methods MDR-TB kit and DNA sequencing were in concordance. Based on DNA sequencing resistant strains mostly showed the precense of mutation in codon 531 and 315 for *rpoB* and *kat*G 315 respectively.
To develop rapid diagnostic methods for tuberculosis especially for drug resistant patients is still an important and challenging problem for TB control programs. In conclusion with this study we try to understand mechanism of resistance and make contrubiton to help rapid diagnosis and treatment of MDR-TB cases.

P 204

Phenotypic and genotypic characterization of resistance to bedaquiline and delamanid in *M. tuberculosis* clinical strains

Simone Battaglia[1], Emanuele Borroni[1], Andrea M. Cabibbe[1], Matteo Chiacchiaretta[1], Maria Rosaria De Filippo[2], Faisal Masood[3], Sabira Tahseen[3], Daniela Maria Cirillo[1], Cryptic Consortium[4]
[1]Emerging Bacterial Pathogens Unit, Division of Immunology, Transplantation and Infectious Diseases, IRCCS San Raffaele Scientific Institute, Milan, Italy
[2]Emerging Bacterial Pathogens Unit, Division of Immunology, Transplantation, and Infectious, Diseases; Center for Translational Genomics and Bioinformatics, IRCCS San Raffaele Scientific Institute, Milan, Italy
[3]National Tb Reference Laboratory, Islamabad, Pakistan
[4]Nuffield Department of Medicine, John Radcliffe Hospital, University of Oxford, Oxford

The increasing incidence of multi- and extensively- drug resistant tuberculosis (M/XDR-TB) represents a serious public health problem. Among new antituberculars for TB treatment, bedaquiline (BDQ) and delamanid (DLM) are the two most promising drugs. The molecular basis of resistance to these drugs is only partially understood. Mutations responsible for resistance to BDQ are found in *atpE*, which encodes for the ATP synthase c-F0 subunit and in *Rv0678*, a regulator which controls expression of MmpS5-MmpL5 efflux pump. Recently, it was demostrated that also mutations in *pepQ* gene could be involved in BDQ-R phenotype. Mutations leading resistance to DLM were found in *Rv3547* and in genes involved in F420 biosyntesis pathway. Previous studies demostrated that BDQ and DLM resistance may also occur in M/XDR-TB isolates without exposure to the drug. Therefore, knowledge of the molecular mechanisms leading to BDQ and DLM resistance is fundamental to address promptly the correct therapy. A total of 787 MTBC strains representing sixteen different lineages and all known drug susceptibility profiles were included in the study: minimal inhibitory concentrations (MICs) of BDQ and DLM were determined by using novel microtiter plates (UKMYC5, Thermo Fisher). MIC values of BDQ-R and DLM-R strains were confirmed by Bactec 960 MGIT system (BD) and resazurin microtiter assay (REMA) as reference methods. Genomic DNA was extracted by CTAB method for Whole Genome Sequencing (WGS) analysis. Paired-end libraries were prepared using Nextera XT DNA Sample Preparation kit and sequenced on NextSeq 500 platform (Illumina Inc). Samples showing a coverage of ≥ 30X were considered for SNPs calling performed by PhyResSE web-tool and the MTbseq pipeline. MIC values obtained by a microtiter plates revealed 7 BDQ-R and 4 DLM-R, confirmed by reference methods. WGS analysis on these strains revealed the presence of 7 non-synonymous mutations in the genomic loci investigated. Of the 7 BDQ-R strains only 5 harboured mutations in the candidate gene *Rv0678* (Arg38fs, 2 Ile67fs, Leu136Pro, Gly121Arg). The analysis of the 4 DLM-R strains genomes revealed the presence of 3 mutations in *ddn* gene (Trp20STOP, Leu90fs, Pro5fs) in 3 strains and the absence of mutations in the last DLM-R isolate. The analysis of susceptible strains revealed the presence of others non-synonymous mutations which do not correlate to drug resistance. The results of this genotypic-

phenotypic correlation allowed to identify and discriminate SNPs correlated to DR phenotype from SNPs without any DR correlation. The final goal is to obtain an encyclopaedia of characterized mutations to identify DLM and BDQ resistant strains by WGS analysis. Finally, it is worth nothing that the population of strains were isolated from patients never exposed to DLM or BDQ, confirming that mutations inducing resistance phenotype are pre-existing in MTBC circulating strains.

P 217

pncA sequence analysis for confirmation of PZA resistance

Elvira Richter[1], Ulrich Eigner[1], Ingelore Frischmann[1], Anke Veldenzer[1], Michael Weizenegger[1]
[1]Labor Limbach, Tuberculosis, Heidelberg, Germany

Pyrazinamide (PZA), one of the first line drugs for TB treatment, is active only at low pHs *in vivo* as well as *in vitro* for performing drug susceptibility testing (DST). The broth based Bactec MGIT 960 technique using a particular, acidified medium is accepted as best performing method for DST of PZA. However, false resistant results are known to occur. Thus, resistant PZA results have to be verified, usually by repetition of the DST assay. As alternative, mutation analysis of the *pnc*A gene, which encodes the enzyme pyrazinamidase, responsible for activation of the prodrug PZA, is recommended to confirm true resistance to PZA.
In our laboratory, we assessed the *pnc*A gene sequences in all strains tested resistant to PZA by phenotypic drug susceptibility testing to estimate the presence and distribution of *pnc*A mutations in PZA-resistant *M. tuberculosis* strains isolated in a German TB laboratory. A set of PZA susceptible strains were used as controls.
We included 55 TB strains, including 26 PZA susceptible and 29 PZA resistant strains. We found a great variety of different patterns of genetic variants scattered over the full length of the gene. Most alterations were single base mutations leading to amino acid changes, but also resulting in a stop codon. Deletion of single nucleotides could be determined, leading to frame shifts. In one patient, a large genome fragment comprising nine genes including *pnc*A was deleted, which could only be confirmed by next generation sequencing. For three strains, no genetic alteration could be detected (one of them was determined as intermediate resistant to PZA). In some resistant strains, an additional (lineage specific) silent mutation could be documented, which also was present in some of the susceptible strains. Analyzing PZA-susceptible strains (first line drugs susceptible and resistant) we found none of the mutations detected in PZA resistant isolates.
In conclusion, by sequence analysis of the *pnc*A gene of strains phenotypically resistant to PZA, we could confirm the high variation of genetic alterations in PZA-resistant strains isolated in Germany. Additional sequencing of the *pnc*A gene in cases of phenotypic PZA drug resistance can improve diagnostic validity of drug susceptibility testing of the still important first line drug PZA. However, sequence data have to be analyzed carefully to adequately appraise any mutation detected.

P 220

Detection of low-frequent resistance-mediating variants in tuberculosis bacteria using next-generation sequencing

Viola Schleusener[1], Ivan Barilar[1], Silke Feuerriegel[2], Stefan Niemann[2]
[1]Molecular and Experimental Mycobacteriology, Research Center Borstel, Germany
[2]Molecular and Experimental Mycobacteriology, Research Center Borstel; German Center for Infection Research (DZIF), Borstel Site, Borstel, Germany

Increasing numbers of multidrug-resistant tuberculosis (TB) threaten global TB control. Solid molecular resistance analysis is challenged by the heterogeneity of the infecting bacterial populations, comprising both susceptible and resistant cells. This phenomenon, termed heteroresistance, describes the concurrent presence of wild-type and resistance-conferring alleles (in one patient). Whole genome sequencing using next-generation sequencing (NGS) approaches has the potential to simultaneously detect wild-type and mutant alleles. However, based on recent publications the proportion of mutant alleles must reach the threshold of 30% to be detected confidentially.
To address this challenge we implemented a NGS variant detector which not only detects but also statistically rates mutations in resistance-mediating genes considering inter alia base quality scores and frequency of the alternate allele. As training dataset we generated artificial sequencing files containing different resistance conferring mutations at varying proportions ranging from 1% to 30% and coverages from 50 to 500 times. This first simulation gave us the theoretical detectable threshold of 2% mutant

allele frequency with at least 400 reads coverage using this approach.
We next evaluated this method with real sequencing data and generated 6 artificial mixtures by blending DNA from one susceptible mycobacterial strain and two different rifampicin mono-resistant strains (1%, 5% and 10%). The samples where sequenced using 500 times coverage on an Illumina MiSeq system. This yielded 100% sensitivity and specificity to detect the *rpoB* mutations in all proportions tested, even for 1% resistant bacteria. We could also show good performance in detecting resistance-conferring mutations with alternate allele frequency up to 5%.
Reliable molecular detection of low-level resistance mutations is an important cornerstone to inform individualized and precise TB therapy. The here presented method is beneficial to render NGS-based diagnostics more sensitive to the presence of heteroresistance and can be implemented in automated and end-user friendly analysis tools of genomic data.

P 221

Evaluation of Next Generation Sequencing library preparation kits based on enzymatic fragmentation for medium and high throughput whole genome analysis of clinical *M. tuberculosis* complex strains

Vanessa Mohr[1], Tanja Ubben[1], Carina Hahn[1], Tanja Struve-Sonnenschein[1], Anja Lüdemann[1], Christian Utpatel[1], Thomas A. Kohl[1], Stefan Niemann[2]
[1]***Molecular and Experimental Mycobacteriology, Research Center Borstel, Germany***
[2]***Molecular and Experimental Mycobacteriology, Research Center Borstel; German Center for Infection Research (DZIF), Borstel Site; National Reference Center for Mycobacteria, Research Center, Germany***

The Next Generation Sequencing (NGS) technology allows for a paradigm change in diagnostics and genotyping for individualized treatment, outbreak investigations, longitudinal molecular epidemiological studies, and TB surveillance. It facilitates the rapid analysis of nearly complete genomes of clinical *Mycobacterium tuberculosis* complex (MTBC) isolates. Therefore, NGS based whole genome sequencing (WGS) analysis goes far beyond conventional molecular tests used for drug susceptibility testing by being able to interrogate nearly the whole genome for variants involved in resistance development (resistome analysis).
The introduction of benchtop sequencing instruments and reduction of sequencing costs is leading now to the use of WGS as routine tool in diagnostics and has been shown to advance treatment and surveillance of Tuberculosis (TB). Still, TB remains one of the ten leading causes of death worldwide while we are faced with intimidating numbers of multi-drug-resistant (MDR) or nearly untreatable extensively drug-resistant (XDR) strains. The basis for new concepts to improve patient care through faster and comprehensive resistance profiling using WGS requires thorough quality control and assessment, from sequencing material preparation to sequence run on different NGS instruments and data analysis to ensure highest quality of results for best possible decision making.
Along with the widespread application of NGS, the market for library preparation kits diversified with an increasing number of commercial kits offering a wide range of options for factors like price, ease of use, hands-on time and required input material. Here we compare a range of NGS Kits and methods based on enzymatic fragmentation from different companies for Illumina sequencing of MTBC samples. We systematically evaluated input range, hands-on time, simplicity, costs, fragment distribution, and resulting sequence information. This comparison can act as a guideline especially for novel users to determine which kit is the most appropriate and efficient for their applications according to their requirements.

P 223

Targeting *Mycobacterium tuberculosis* in the blood of patients with active and latent tuberculosis

Stefan V. Panaiotov[1], Vladimir Milanov[2], Maria Nikolova[1], Elena Nikolova[3], Rumen Dimitrov[4], Reni Kalfin[5]
[1]***National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria***
[2]***University Hospital for Respiratory Diseases "St. Sofia", Sofia, Bulgaria***
[3]***Institute of Experimental Morphology, Pathology and Anthropology, Bulgarian Academy of Sciences, Sofia, Bulgaria***
[4]***Faculty of Physics, Sofia University, Sofia, Bulgaria***
[5]***Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria***

Background: A third of the world's population has latent infection with *Mycobacterium tuberculosis*. The tuberculosis reservoir in areas

of low endemicity are individuals with latent TB. The factors and moment of reactivation of latent bacilli remain unknown. Little is known about the location of the bacilli in latently infected individuals. Long-term mycobacterial persistence in the lungs has been reported, however persistence in the blood has not been investigated. Hypothesis for the existence of latent *M. tuberculosis* in the blood have been arisen and detection of *M. tuberculosis* in peripheral blood as a diagnostic tool has been applied.

The aim of our study was to target *M. tuberculosis* in the blood of patients with active and latent tuberculosis by blood culturing resuscitation and NGS DNA sequencing.

Results: Twenty eight blood samples of healthy individuals, 16 of patients with active TB, 4 with latent TB and 10 with other diseases were studied. Several culture media were tested. Blood microbiota resuscitation was performed in BHI broth supplemented with vitamin K 1 mg/ml, 2% sucrose, 0.25% sodium citrate and 0.2% yeastolate at 43^0C for 72 h. All tested blood samples were culture positive, as confirmed by Gram staining and TEM. TEM images demonstrated well defined cell structures. Analysis for bacterial and eukaryotic species was performed by 16S rRNA and ITS2 targeted sequencing. The obtained sequences were clustered (≥97% identity) in Operational Taxonomic Units (OTUs). Among cultured and uncultured samples we identified OTUs similarity with 47 bacterial orders, belonging to 15 phyla (including family *Mycobacteriaceae*) and 39 fungi orders blonging to 2 phyla. Blood-group differences were identified among the bacterial microbiome composition. The preliminary results of the on-going experiments demonstrate that the dynamics of the blood microbiota could be applied to monitor latent TB reactivation.

Conclusion: Rich blood microbiome biodiversity is innate of the healthy individuals. Optimization of resuscitation *M. tuberculosis* culture media and conditions are on-going. Interventional strategies to bind the host blood latent *M. tuberculosis* with the states of health and disease are a research goal.

P 33

DevS/DosS sensor is bifunctional and its phosphatase avtivity percludes aerobic DevR/DosR expression in *Mycobacterium tuberculosis*

Priyanka Kumari[1], Kohinoor Kaur[1], Saurabh Sharma[1], Snigdha Sehgal[1], Jaya Tyagi[1]
[1]Department of Biotechnology, All India Institute of Medical Sciences, New Delhi, India

Two-component systems, comprising histidine kinases and response regulators, empower bacteria to sense and adapt to diverse environmental stresses. Some HKs are bifunctional; their phosphorylation (kinase) and dephosphorylation (phosphatase) activities towards their cognate RRs permit the rapid reversal of genetic responses to an environmental stimulus. DevR-DevS/DosR-DosS is one of the best-characterized TCS of *Mycobacterium tuberculosis*. The kinase function of DevS is activated by gaseous stress signals, including hypoxia, resulting in the induction of ~48-genes DevR dormancy regulon. Regulon expression is tightly controlled and lack of expression in aerobic Mtb cultures is ascribed to the absence of phosphorylated DevR. In this study, we show that DevS is a bifunctional sensor and possesses a robust phosphatase activity towards DevR. We used site-specific mutagenesis to generate substitutions in conserved residues in the Dimerization and Histidine phosphotransfer domain of DevS and determined their role in kinase/phosphatase functions. *In vitro* and *in vivo* experiments, including a novel *in vivo* phosphatase assay, collectively establish that these conserved residues are critical for regulating kinase/phosphatase functions. Our findings establish DevS phosphatase function as an effective control mechanism to block aerobic expression of the DevR dormancy regulon. Asp-396 is essential for both kinase and phosphatase functions, whereas Gln-400 is critical for phosphatase function. The positive and negative functions perform opposing roles in DevS: the kinase function triggers regulon induction under hypoxia, whereas its phosphatase function prevents expression under aerobic conditions. A finely-tuned balance in these opposing activities calibrates the dormancy regulon response output.

P 66

Tuberculosis in a South African correctional centre: High mobility of detainees impedes TB control

Katie Baird[1]
[1]Department of Clinical Microbiology and Infectious Diseases, University of the Witwatersrand, Johannesburg, South Africa

Correctional centres provide an environment conducive to ongoing tuberculosis (TB) transmission and disease progression. South Africa has the third highest incidence of TB globally and one of the highest incarceration rates globally. The combination of a high TB incidence and a high number of people detained means that South African correctional centres are a significant TB reservoir.
The South Africa government has adopted measures to tackle TB inside the correctional centres. Detainees are screened for TB upon admission, biannually and on release. Since 2013, onsite GeneXpert testing has been implemented in correctional centres. Treatment is provided to all detainees diagnosed with TB. While the South African correctional system has established diagnostic and treatment programs, little information is known about TB treatment completion in South African correctional centres.
This study was a prospective observational study that enrolled TB-positive detainees over a 7-month period from a correctional centre located in the Western Cape of South Africa. Ninety male adult detainees, who were diagnosed with TB by the correctional health services, provided informed consent and were enrolled in the study.
A sputum sample was collected at treatment initiation from each participant for smear microscopy and culture. Participants were traced for 6-months following treatment initiation to determine at what stage of treatment they exited the correctional centre.
74% (n = 67) of sputum samples were culture positive. The smear positivity rate among culture positive cultures was 66%. There was high mobility of participants. 50% (N = 45) of participants returned to their communities before completing the intensive phase of treatment. Only 13% (n = 12) of participants completed treatment at the correction centre.

The high smear positivity rate combined with the high mobility of detainees is a concern for TB control efforts. Successful treatment completion is a vital component of TB infection control. Detainees with infectious tuberculosis, who are released before treatment completion, may play an important role in TB transmission. Strengthening linkages between the correctional TB services and community TB services are necessary to ensure that detainees complete TB treatment after release and to prevent TB transmission.

P 67

Migration and tuberculosis in the Czech Republic

Maria Müllerová[1]
[1]***Department of Mycobacteriology, Citylab Ltd., Prague, Czech Republic***

Czech Republic (CZ) situated in Central Europe is at the crossroads for migrants and immigrants. The majority does not seek asylum here and moves on. However, these people come from countries with high incidence of tuberculosis (TB), such as those of the former Soviet Union. TB together with AIDS and malaria are are the most serious infectious diseases in the world. TB affects the whole world and so the World Health Organisation (WHO) declared it a global threat and its occurrence is subject to obligatory notification.
In CZ regarding the number of newly notified cases and relapses of TB in recent years the situation is favourable so far, the incidence being around 5 per 100,000 inhabitants: in 2015 – 518 (4.9) of which 110 were foreigners; in 2016 – 515 (4.9) including 150 foreigners; in 2017 – 512 (4.9), the final number will be available April 30, 2018.
More than 1/3 of the global population is infected with TB creating enormous reservoir of TB infection. Although the infection is usually latent, manifest disease develops in 5 – 10% of which 490,000 cases are MDR-TB. The European Centre for Disease Control (ECDC) determined 13 priority infectious diseases, of which MDR-TB occupies first place.
Migration to Europe culminated in 2015 and led to increased incidence of TB and its MDR forms. This in the future may negatively influence the epidemiologic situation in CZ. West European statistics show that up to 30% immigrants are acutely or chronically ill. The Czech Ministry of Interior informed: “In 2015 among 3350 examined illegal migrants and asylum seekers TB was detected in 3 cases, in 2016 one case out of 1741 examined was found, in 2017 there wer 5 cases among 1870 examined persons.”
So far the European migration crisis did not affect CZ. However, no measure to ward off a possible deterioration of the present situation can be neglected. Therefore the same set of examination techniques as recommended by WHO should be readily available, namely chest x-rays, IGRA tests, mostly QFN. In positive QFN and cases with respiratory difficulties sputum is to be examined by the GeneXpert MTB/RIF, the quickest and most efficient molecular biologic method. See Dara M., de Colombani P., Petrova-Benedict R. et al.: Minimum package for cross-border TB control and care in the WHO European region: a Wolfheze consensus statement. Eur. Respir.J.2012 Nov.40(5):1081-90.

P 111

TB in indigenous peoples with settlement at puerto narino- Amazonas, Colombia

Francy Johanna Perez Llanos[1], **Alejandro Vega Marín**[2], **Luz Mila Murcia**[3], **Clara Viviana Mape**[2], **Carlos A. Parra-López**[2], **Myriam Navarrete**[2], **Ricardo Sánchez**[4], **Stefan Niemann**[5], **Martha I. Murcia**[2]
[1]***Molecular and Experimental Mycobacteriology, Research Center Borstel, Germany; Grupo Micobacun, Departamento de Microbiología, Facultad de Medicina, Universidad Nacional de Colombia, Bogotá, Colombia***
[2]***Grupo Micobacun, Departamento de Microbiología, Facultad de Medicina, Universidad Nacional de Colombia, Bogotá, Colombia***
[3]***Laboratory of Public Health, Amazonas, Colombia, South America***
[4]***Department of Psychiatry, School of Medicine, Universidad Nacional de Colombia. South America; Molecular and Experimental Mycobacteriology, Research Center Borstel, Germany***
[5]***Molecular and Experimental Mycobacteriology, Research Center Borstel, Germany***

Introduction: In Colombia, 12,918 cases of tuberculosis were reported in 2015 for a national incidence of 24.2 cases / 100000 inhabitants, Amazonas had the highest incidence of 72.1 cases / 100.000 inhabitants. 5.3% of these cases belong to the indigenous population.
Indigenous peoples have the greatest risk of tuberculosis transmission due to the limited access to health services, poverty, malnutrition, overcrowding, difficult geographical access and mainly cultural beliefs. Those features contribute to get a late diagnosis and treatment, promoting

a permanent cycle of TB infection. The aim of this study was to determine the current state of active tuberculosis at the municipality of Puerto Nariño, Amazonas, Colombia and to get a first insight of the *Mycobacterium tuberculosis* complex (MTBC) population structure.
Methods: In 2016, an active search of respiratory symptomatic subjects was carried out on 6309 individuals through medical consultation. Smear microscopy, BACTEC™ MGIT™ and Loewenstein Jensen cultures were performed on sputum samples.
MTBC isolates were identified thought MTP 64 Ag and evaluated through MGIT™ SIRE (BD), GeneXpert® (Cepheid) and GenoType®MTBDR plus 2.0 (Hain). The population structure was analyzed by Spoligotyping and 24 Loci MIRU VNTRtyping.
Results: In total, 808 out of 6309 individuals investigated were identified as respiratory symptomatic subjects. 80 (10%) patients were diagnosed with TB; whereas only 20 cases (25%) had a positive AFB smear, Only 74 (92.5%) MTBC isolates were recovered. 73 isolates (98%) were sensible for Rifampicin (RMP) and Isoniazid (INH). One isolate (1, 3%) showed resistant against INH and RMP (multidrug resistant TB). Phylogenetic analysis revealed 73 isolates belonging to lineage 4 (Euro-American lineage), with a predominance of the LAM (Latin American Mediterranean) sublineage (38, 52.7%). LAM6 37/74 (50%), LAM9 2/74 (2.7%), and T2 34/74 (45.9%) genotypes were dominant. One isolate had an orphan pattern.
Conclusions: These findings revealed that TB incidence was 2 times higher than that was reported in 2015 for Amazonas along with a Low MDR rate. Euro American Lineage seems to be widely spread in Colombia even in isolated settings. Higher resolution is needed to understand phylogeny and transmission in future studies.

P 136

Xpert MTB/RIF assay useful for pediatric patient tuberculosis disease diagnosis

Virginia Lora-Téllez[1], María Lucía Pérez-Ricardez[1], Maricruz Gutiérrez-Brito[1]
[1]Hospital Para el Niño Poblano, Puebla, Mexico

Introduction: Diagnosis tuberculosis in children is difficult as children are less likely to have obvious symptoms of pulmonary tuberculosis, besides the incidence for extra-pulmonary sites such as lymphatic and meningeal tuberculosis, are more common in young children. Samples such as sputum (gastric lavage fluid), cerebrospinal fluid and lymphatic ganglion are more difficult to collect too. Even when these samples can be collected, it may have very few tuberculosis bacteria in it (paucibacillary smear-negative disease). The Xpert MTB/RIF assay is a nucleic acid amplification (NAA) test that uses a disposable cartridge with the GeneXpert Instrument System. The test simultaneously detects *Mycobacterium tuberculosis* complex (MTBC) and resistance to rifampin (RIF) in less than 2 hours.
Material and method: The investigational assay was used to test gastric lavage fluid, sputum, bronchial lavage, cerebrospinal fluid and lymphatic ganglion samples from 231 pediatric patients with tuberculosis disease presumptive diagnosis. All the collected samples were available for smear with microscopy for acid-fast bacilli, culture for mycobacteria, and for the Xpert MTB/RIF assay.
Results: This clinical study included 231 pediatric patients; female 53.2%, male 46.8%. Age groups affected 0-5 years: 56.75%, 6-15 years: 40.75% and > 15 years: 2.6%. 17 patients had tuberculosis disease (7.35%), 8 patients had pulmonary tuberculosis, 4 lymphatic tuberculosis, 3 disseminated tuberculosis and 2 meningeal tuberculosis. The Xpert MTB/RIF assay detected 16 of 17 cases for MTBC and one of these for resistance to rifampin, failure MTBC detected once for a ganglion sample. Culture for mycobacteria was positive in 16 of 17 cases, failure once for a gastric lavage fluid sample. Smear with microscopy for acid-fast bacilli was effective in 10 of 17 cases for tuberculosis. All the collected samples were paucibacillary.
Conclusion: The standard cultures can take 2 to 6 weeks for MTBC to grow and conventional drug resistance test can add 3 more weeks, but the Xpert MTB/RIF assay results are available less than 2 hours, and this information provided by the Xpert MTB/RIF assay aids in selecting treatment regimens and reaching infections control decisions quickly. However, the Xpert MTB/RIF assay does not replace the need for smear with microscopy for acid-fast bacilli, culture for mycobacteria, and growth-based drug susceptibility testing.

P 156

Association of Expression of Negative Regulators of Human Innate Immune Response with Susceptibility to Tuberculosis in Turkish Population

Emel Eker[1], Togrul Nagiyev[1], Begum Kayar[2], Ali Üçkayabaşı[1], Gülfer Yakıcı[1], Firat Karsli[1], Fatih Koksal[1]
[1]Department of Medical Microbiology,

Medical Faculty, Cukurova University, Adana, Turkey
[2]Tropical Diseases Research and Application Center, Cukurova University, Adana, Turkey

Tuberculosis (TB) is one of the leading causes of infectious death worldwide, yet its immune pathogenesis remains incompletely understood. The innate immune system is critical for the initial host defense against mycobacteria. It has been proposed that host immunogenetic risk factors play an important role in the pathophysiology of tuberculosis and susceptibility to tuberculosis as well as in many chronic infections. In this context, few molecular studies have indicated that expression levels of negative regulator genes of human innate immune response were associated with an occurrence of tuberculosis in different human populations.
In this study we aimed to investigate the potential role of gene expression levels of negative regulators of human innate immune response on susceptibility to tuberculosis in Turkish population.
Two groups, 20 patients with active pulmonary tuberculosis and 20 healthy volunteers, were enrolled in the study. The diagnosis of TB in sputum samples were verified by phenotypic and genotypic methods. Mononuclear cells were separated from the fresh blood samples. The mRNA levels of negative regulator genes in peripheral-blood-mononuclear-cells (PBMCs) were investigated by using quantitative Real-Time PCR. The results were analysed using double delta Ct analysis.
According to the results of our study, gene expression levels of IL-1 receptor-like 1 (ST2), Toll-interacting protein (TOLLIP) and single immunoglobulin IL-1R-related molecule (SIGIRR, TIR8) were increased (the fold differences were 7,19; 6,046 and 1,6, respectively) in patient group, whereas mRNA of IL-1 receptor associated kinase (IRAK)-M gene was extremely decreased (the fold difference was 0,052).
İn conclusion, we assumed that these regulator genes might be strong biomarkers when searched with other biomarkers such as microRNAs.
Key Words: Negative regulator genes of immune response, Real-Time PCR, Tuberculosis.

P 195

Cutaneous non-tuberculosis mycobacterial infections: A six year Retrospective Evaluation

Gülnur Tarhan[1], Hulya Simsek[2]
[1]Adıyaman University, Faculty of Medicine, Department of Medical Microbiology, Adıyaman, Turkey
[2]Ministry of Health, Generel Directorate of Public Health, National Tuberculosis Reference Laboratory, Ankara, Turkey

Objective: Cutaneous non-tuberculosis mycobacterial (NTM) infections are rarely seen compared to other bacterial fungal pathogens. These infections may develop depend on surgery, traumatic injury, cosmetic procedures, seconder infection or some immunosuppressed conditions. There are limited studies on the incidence and prevalence of cutaneous NTM infections. The aim of this study was to investigate the frequency of tuberculosis and non-tuberculosis mycobacteria in biopsy specimens.
Materials and Methods: Twenty six skin biopsy specimens were evaluated between January 1, 2004 and December 31 2010 at National Tuberculosis Reference Laboratory by using microscopy, solid (Löwenstein jensen culture) and liquid medium (MGIT 960) and real time PCR (Artus®M. tuberculosis RG Real Time PCR, Valencia, Calif.).
Results: Three specimen were positive with microscopy culture, but negative with PCR test. One specimen was positive with microscopy, culture and PCR. When four positive isolates were tested by PCR-RFLP test, two isolates were identified as M abscessus, one as M. cheloneae and the other as M fortuitum.
Key Words: Cutaneous non tuberculosis, M abscessus, M. cheloneae, M fortuitum.

P 196

A retrospective evaluation of Mycobacterium abscessus prevelance in pulmonary diseases

Gülnur Tarhan[1], Hulya Simsek[2], Ismail Ceyhan[3]
[1]Adıyaman University, Faculty of Medicine, Department of Medical Microbiology, Adıyaman, Turkey
[2]Ministry of Health, Generel Directorate of Public Health, National Tuberculosis Reference Laboratory, Ankara, Turkey
[3]Atatürk Chest Diseases and Thoracic Surgery Education and Research Hospital, Ankara, Turkey

Objective: *Mycobacterium abscessus* is the most common rapidly growing mycobacterium. It is responsible for a wide spectrum of skin and soft tissue diseases, bacteremia, ocular, lung and other infections. The lungs are the most frequent site of infection, and *M. abscessus* infections progress slowly if left untreated. There are limited data about pulmonary infection depend on *M.*

abscessus. This study was to determine the prevelence of M abcessus in sputum specimens during a 6-year time period.
Materials and Methods: 2537 pulmonary specimens (sputum, *bronchoalveolar lavage,* gastric lavage) were evaluated between January 1, 2004 and December 31 2010 at National Tuberculosis Reference Laboratory by using microscopy, solid (Löwenstein jensen culture) and liquid medium (MGIT 960).
Results: 211(8.81%) specimen were found positive with culture. 205 (95.34%) isolate was identified as Mycobacterium tuberculosis complex with biochemical and rapid test. The other 6 isolate was tested PCR-RFLP. Three isolate (1.4%) was identified as Mycobacterium abscessus.
Conclusion: Mycobacterium abscessus was not commonly detected in pulmanory samples in our study population.
Keywords: Mycobacterium abcessus, pulmonary infection

P 198

Retrospective Evaluation of 38 Patients with Tuberculous Pleurisy in Mersin, Turkey

Mahmut Ülger[1], Nurbanu Kurnaz[1], Seda Tezcan Ülger, Nuran DelialioĞlu[1], Gönül Aslan[1]
[1]***Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Mersin University, Mersin, Turkey***

Objectives: Tuberculosis (TB) caused by the bacillus *M. tuberculosis*, commonly affects the lungs (pulmonary TB) but it can spread other sites of the body (extrapulmonary TB-EPTB). EPTB accounts for ~15% of the all TB cases. Tuberculous pleurisy (TP) is the second most common form of EPTB after TB lymphadenitis and in developing countries it is one of the major causes of exudative pleural effusion, with geographically varying rates between 4-25%. In this study, we aimed to evaluate the mycobacteriological and histopathological results and demographic data of TP cases and to determine the anti-TB drug susceptibility patterns of these mycobacterial strains.
Methods: Between January 2006 and February 2018, 762 cases with suspected to TP were retrospectively evaluated. By Ehrlich Ziehl-Neelsen staining method acid fast bacilli (AFB) positive cases, in culture method *M. tuberculosis* complex (MTC) isolated cases and histopathologically diagnosis as TP cases were included in this study.
Results: *Of the 762 cases with suspected TP, 38 cases (5%) were diagnosed as TP with microbiological and histopathological evaluations. Of these cases, 22 (57.9%) were male, 16 (42.1%) were female and the mean age was 43 (2-82) years. Of the 38 TP cases, 31 (81.6%) cases were culture positive, seven (18.4%) cases were AFB positive and chronic granulomatosis inflammation was detected in 30 (78.9%) cases with histopathological examination. In two (6.5%) cases isoniazid (INH) resistance, in one (3.2%) case streptomycin (SM) resistance, in one (3.2%) case both SM and INH resistance was detected in culture positive 31 cases. Rifampicin and ethambutol resistance was not detected in the isolates. In the remaining 27 (87.1%) cases, primary anti-TB drug resistance was not detected. The most common symptoms among the 37 (97.3%) cases, whose resume and clinical findings could be accessed, were respiratory disorder, cough and chest pain respectively. One (2.6%) case had previous history of TB, three (7.9%) cases had domestic TB history (close contact) and two (5.3%) cases had asbestos exposure.*
Conclusion: *Pleural sample culture that is used for diagnosis of TP, is a reliable and indispensable method in terms of growth of bacilli and determination of drug sensitivity patterns. Additionally, diagnostic efficiency increases significantly when the pleural samples are evaluated both with microbiological and histopathological methods.*
Keywords: *Tuberculosis, Tuberculous pleurisy, Culture, Histopathology*

P 3

Bio-guided Isolation of Anti-Mycobacterium ulcerans Principles from Allanblackia kisonghi Vermoesen (Clusiaceae)

Patrick Valere Tsouh Fokou[1], Regina Appiah-Opong[2], Dorothy Yeboah-Manu[3], Phyllis Addo[2], Abena Adoma Kissi-Twum[2], Lauve Rachel Tchokouaha Yamthe[4], Dorcas Osei-Safo[5], Fabrice Boyom[6], Alexander K. Nyarko[2]
[1]University of Yaounde 1, Cameroon
[2]Noguchi Memorial Institute for Medical Research, Accra, Ghana
[3]Noguchi Memorial Institute for Medical Research, Bacteriology, University of Ghana
[4]Institute of Medical Research and Medicinal Plants Studies, Yaounde, Cameroon
[5]University of Ghana
[6]Antimicrobial Agents Unit, LPMPS, Department of Biochemistry, University of Yaounde 1, Cameroon

Buruli ulcer (BU) is the third most prevalent human mycobacteriosis, and West Africa accounts for 90% of the global disease burden. The recommended drugs, rifampicin based combination therapies are neither affordable nor accessible to a large proportion of the afflicted population who thereby rely mainly on herbal remedies. *Allanblackia kisonghi* Vermoesen is used for treating various skin infections and some microbial diseases in traditional medicines. In order to validate these ethnobotanical practices, the *Allanblackia kisonghi* fruit was investigated for its ability to inhibit growth of *Mycobacterium ulcerans*. An *in vitro* bio-guided fractionation was used to isolate the active principle of *Allabiankia kisongii* using solvent partitioning and extensive column chromatography techniques. Spectroscopic methods (ESI-MS, ^{1}H-NMR, ^{13}C-NMR) was used to elucidate the structure of the active compounds. Extracts were tested against *Mycobacterium ulcerans* strain ATCC 19423 and in-house reference clinical isolate, NM209 using the Resazurin Microtiter Assay. The antimycobacterial activity of the extracted compounds and Rifampicin, were evaluated using the *checkerboard assay*. Furthermore, the extracts were investigated for their toxicity against Chang liver cells using the MTT assay. Overall, the bio-guided fractionation led to the isolation of two bioflavonoids, Morelloflavone and Fukugeside, that showed promising anti-*M. ulcerans* activity, with minimum inhibitory concentrations of 16-32 µg/mL. Both compounds were weekly toxic to Chang liver cells with IC_{50} values of 125.4->128 µg/mL. Fukugeside also showed ability to potentiate Rifampicin activity against *M. ulcerans* with a Fractional Inhibitory Concentration Index (ΣFIC) of 0.44. Thus, these results suggested that Morelloflavone, and Fukugeside have the potential to be developed as potential antimycobacterials in the treatment of Buruli ulcer. However, further studies have to be performed to describe their safety and efficacy *in vivo.*

P 13

Immunogenicity of the *Mycobacterium tuberculosis* aldehyde dehydrogenases family proteins following stimulating MDRTB patients naive T-cell

Alireza Hadizadeh Tasbiti[1], Shamsi Yari[1], Morteza Ghazanfari[1], Morteza Masomi[1], Farid Abdolrahimi[1], Sharareh Khanipour[1], Shahin Pourazar[1], Seyed Davar Siadat[1]
[1]Tuberculosis and Pulmonary Research Department, TB Protein Chemistry Laboratory, Pasteur Institute of Iran, Tehran, Iran

Drug-resistant TB is a continuing threat. In 2016, there were 600 000 new cases with resistance to rifampicin (RR- TB), the most effective first-line drug, of which 490 000 had multidrug-resistant TB (MDR-TB). Most deaths from TB could be prevented with early diagnosis and appropriate treatment. A reliable TB biomarker is urgently needed to monitor the response to treatment in MDRTB patients. The present study has employed proteomic approach, to determine the role of T cells in MDRTB patients against *M.tuberculosis* purified aldehyde dehydrogenase proteins (Rv0147) in comparison with healthy contacts. MDRTB Proteins were prepared by detergent phase separation and saturated ammonium sulfate. 2DE was performed using the isoelecteric focusing system (Ettan IPGphor 3). Protein sequences were recovered from *Mycobrowser* server (https://mycobrowser.epfl.ch) followed by Mass spectrometry MALDI-TOF. Five days after starting dendritic cells culture from MDRTB patients PBMC's and autologous T cells were isolated via Pan T cell selection and pulsing dendritic cells with aldehyde dehydrogenase proteins. The *cytokines measured* include Th1-

cell cytokines (gamma interferon) IFN-γ and IL-10. The purified protein, Rv0147, stimulated PBMC from patients with MDR *tuberculosis* produced significantly higher levels of INF- than did those from healthy contacts. The purified protein, Rv0147 directly stimulate INF- production by T cells. The purified aldehyde dehydrogenase proteins (Rv0147) and certain of its epitopes directly *stimulate* IFN-γ production by T cells from patients with MDR tuberculosis. As result of potent vaccine should response to induce robust CD8 and *Th1-type* immune responses and at the same time avoid the induction of immune tolerance, *these results suggest* that the purified protein, Rv0147 *may stimulate Th1-type protective* cytokine responses in MDR TB patients. Thus, next progress in vaccine development will severely rely on identifying potent epitopes with reduced side effects. This present research may allow the identification of some valuable vaccine and drug target candidates.

Key words: Multi drug resistance TB, *cytokines, TB biomarker, TB* purified protein, Dcs

P 38

Mycobacterium tuberculosis cell wall: The potential target for the discovery of new therapies

Chitra Rani[1], Inshad Ali Khan[2], Sujata Sharma[1], Tej Pal Singh[1]
[1]***Department of Biophysics, All India Institute of Medical Sciences, New Delhi, India***
[2]***Clinical Microbiology Division, Indian Institute of Integrative Medicine, New Delhi, India***

Introduction: Tuberculosis (TB) is third heinous disease responsible for the engulfment of human lives. It is a chronic illness caused by *Mycobacterium tuberculosis (M.tb)*. The World Health Organization (WHO) has reported that there were 10.4 million new cases and 1.3 million deaths from TB. Human immunodeficiency virus (HIV) has fueled the TB epidemic. Given the increased incidence of drug resistant TB cases worldwide, there is a desperate need for the development of new anti-tubercular agents that operate via novel modes of action to the currently employed drugs.
Background: Peptidoglycan is an essential component of the cell wall of bacteria including *M.tb*. UDP-N-acetylglucosamine enolpyruvyltransferase (MurA) catalyzes the transfer of an enolpyruvyl group from phosphoenolpyruvate (PEP) to UDP-N-acetylglucosamine (UNAG) to form UDP-N-acetylglucosamine enolpyruvate (UNAGEP), the first committed step in the biosynthesis of *M.tb* cell wall. *M. tb* MurA is encoded by the *murA* (Rv1315) gene, which is made up of 1257 base pairs, producing a protein of 45.50 kDa with 418 amino acids. Since, this metabolic pathway does not exist in mammalian cells. Therefore, Mur A may be a potential target to develop anti-tuberculosis drugs.
Methods: In this study, whole cell active compounds from diverse Chembridge library were used for the virtual screening against MurA *M. tb*. MurA protein was cloned, expressed and purified. Enzymatic activity of *M.tb* MurA was measured by Malachite green colorimetric assay where determination of inorganic phosphate based on the formation of a phosphomolybdate malachite green complex which kept absorbance at 620 nm changed. IC_{50} values of the identified potent inhibitors were calculated.
Results: 700 compounds found active in the whole cell assay (MIC) were screened against MurA of *M.tb*. Few early stage compounds were identified as inhibitors of UDP-N-acetylglucosamine enolpyruvyltransferase activity with IC_{50} values in range of 50-70μM.
Conclusions: Screening of the compounds against enzymatic activity of *M.tb* MurA serves as a promising starting point for the discovery of more potent inhibitors.

P 47

Antimycobacterial efficiency of three essential oils from plant currently used to treat tuberculosis traditionally tuberculosis in Cameroon

Esther Del Florence Moni Ndedi[1], Maximilienne Nyegue[2], Patrick Hervé Betote Diboue[2], Jean Paul Assam Assam[3], Veronique Penlap Beng[4], Fraçois-Xavier Etoa[5]
[1]***University of Yaounde 1, Yaounde, Cameroon***
[2]***Department of Microbiology, University of Yaounde 1, Yaounde, Cameroon***
[3]***University of Yaounde 1, Yaounde, University of Douala, Cameroon***
[4]***University of Yaounde 1, Yaounde; Laboratory for Tuberculosis Research (Ltr), Biotechnology Centre of Nkolbisson, Biochemistry Department, Faculty of Science, Cameroon***
[5]***Department of Biochemistry, Faculty of Sciences, University of Douala, Cameroon***

The designed study carries out an ethnopharmacology survey of medicinal plant used to treat tuberculosis and asses the anti-mycobacterial efficiency of three of

their essential oils plant against four resistant strains of *Mycobacterium tuberculosis*. The ethnopharmacological study was carried out in two localities of Nkam Division where plants specimens were collected. Three plants have been selected and their essential oils were obtained by hydrodistillation and analysed by Gas Chromatography and Gas Chromatography-Mass Spectrometry. Anti-mycobacterial activity of these essential oils was evaluated against four resistant isolates of *Mycobacterium tuberculosis* using the microdilution method. The ethnopharmacology survey showed twelve plants collected and the most used *were Drypetes gossweileri*, *Pentadiplandra brazeana* and *Allium sativum*. Benzyl isothiocyanate was major component in *D. gossweileri* and *P. brazzeana* essential oils at 91.27 % and 96.00 % respectively and linalool at 51.02 % methylallyl trisulfide (12.8%), diallyl trisulfide (11.1%) for *A. sativum*. *A. sativum* and *P. brazzeana* essential oil excibited higher activity with the minimum inhibitory concentrations respective of 78.12 µg/mL and 312.50 µg/mL against extensively resistant isolate while *D. gossweileri* against Isoniazid resistant isolate showed higher activity with MIC of 156.25 µg/mL. The results justify the traditional uses of these plant by Nkam populations for treatment of tuberculosis cases.

Key words: Ethnopharmacological survey**,** Medicinal plants, essential oils, anti-mycobacterial efficiency.

P 59

Preliminary study of the in vitro activity of Tedizolidagainst *Mycobacterium tuberculosis* strains

Pilar Ruiz[1], Manuel Vaquero[2], Manuel Causse del Rio[3], Manuel Casal-Román[4]
[1]Mycobacteria Reference Center, Faculty of Medicine, University of Cordoba, Spain
[2]Faculty of Medicine and Nursing, Cordoba, Spain
[3]Microbiology, Reina Sofia University Hospital, Cordoba, Spain
[4]University of Cordoba, Spain

Introduction: The emergence of multidrug resistant TB strains (MDR), has been a public health threat around the world. According to the WHO, 600 000 new cases of Rifampicin resistance developed in 2016, 490 000 from which were TB-MDR and 190 000 died as a result of MDR-TB.
It is necessary to introduce new antimicrobials in order to fight against TB-MDR. Tedizolid is a novel, potent oxazolidinone drug that interacts with the bacterial 23S ribosome initiation complex to inhibit translation.
Objectives: The aim of this study is to determine the possible in vitro antibacterial activity of Tedizolid against clinical isolates of *Mycobacterium tuberculosis*, including drug-resistant isolates.
Material and methods: The minimum inhibitory activity(MIC) of Tedizolid, was determined against 80 clinical isolates of *Mycobacterium tuberculosis* (TB) (28 sensitive and 52 resistant) from various geographical origins selected from the Mycobacteria Reference Center, Faculty of Medicine, "Reina Sofia " Universitary Hospital, Córdoba, Spain.
To determine Tedizolid MIC against the strains, a Bactec 960 MGIT system (Becton, Dickinson, Sparks, MD) was used with a test concentration range of 0.03 to 16 mg/liter Tedizolid. Briefly, 0,8 ml of an oleic acid-albumin-dextrose-catalase (OADC) enrichment was added to each MGIT culture tube.
Results: All the 80 studied strains were inhibited to concentrations under 1µg/ml. The CMI 90 corresponded to CMI concentrations between 0,5 and 0,25 µg/ml, and CMI 50 between 0,25 and 0,12 µg/ml. There were no statistically significant differences between resistant or sensible strains to first line antitubercular drugs.
Conclusions: Tedizolid is presented as a drug with a high activity. Larger studies are needed to provide definitive data on the efficacy of tedizolid in resistant isolates. It could be an alternative in the treatment of resistant tuberculosis.
This study has obtained economical support and valued substance from MSD.

P 104

Effect of anti-tuberculous drugs on *Mycobacterium tuberculosis* intra-macrophagic behaviour

Charlotte Genestet[1], Florence Ader[2], Fanny Bernard Barret[3], Elisabeth Hodille[4], Gérard Lina[5], Sylvain Goutelle[3], Oana Dumitrescu[6]
[1]CIRI INSERM U1111, University of Lyon, France
[2]Hospices Civils de Lyon - Claude Bernard Lyon 1 University, Infectious Diseases - INSERM U1111, International Center for Research in Infectiology, France
[3]Hospices Civils de Lyon, Université Claude Bernard Lyon 1, France
[4]Microbiology Department, Hospices Civils de Lyon, Hôpital de la Croix Rousse, France
[5]National Reference Center for Staphylococci, Hospices Civils de Lyon, CIRI INSERM U1111, University of Lyon, France
[6]Microbiology, Hospices Civils de Lyon, France

Background: Tuberculosis caused by *Mycobacterium tuberculosis* (MTB) complex remains a deadly infectious disease worldwide. MTB is an intracellular pathogen and autophagy is an essential component in the immune response which leads to tuberculosis clearance. Anti-TB treatment based on isoniazid (INH) and rifampicin (RIF), has evolved in the recent years and new drugs such as linezolid (LNZ) and bedaquiline (BDQ) have been shown to be effective in the management of multidrug-resistant tuberculosis. However, little is known about the impact of these antibiotics on MTB intra-macrophagic behavior, independently of their impact on host cells. The aim of this work was to explore the effect of these four antibiotics on MTB intra-macrophagic behavior.
Materials/methods: MTB was preincubated with INH, RIF, LNZ or BDQ at a sub-inhibitory concentration, or without antibiotics (as control) before macrophages infection. Intramacrophagic MTB survival was evaluated by CFU counting and intramacrophagic MTB behavior, by confocal microscopy, using three markers: anti-MTB antibody (bacterial burden), anti-LC3B antibody (autophagy) and LysoTracker (acidified compartments). To study phagolysosome activation and escape we analyzed the percentage of cells with acidified compartments and percentage of bacteria in acidified compartments. To explore autophagy, we analyzed three criteria: frequency of cells with autophagy compartments, the LC3B puncta count per cells (for autophagy activation), and the frequency of acidified autophagy compartments (for autophagy outcome).
Results: LNZ and BDQ, more than INH and RIF pre-incubation, led to an important decrease of MTB survival during macrophages infection. We showed that INH and BDQ pre-incubation impaired MTB capacity to escape phagolysosome. Pre-incubation of MTB with RIF resulted in an increase of autolysosome formation. LNZ and BDQ pre-incubation led to an increase of autophagy activation and outcome. Thus, the four antibiotics tested act at different levels to affect MTB intra-macrophagic clearance.
Conclusions: Our results suggest that antibiotics that favor autophagy activation and outcome (LNZ and BDQ), would allow a better clearance of MTB by macrophages. These data may provide logical basis for future anti-TB treatment strategies based on autophagy promotion to ultimately improve anti-TB host response.

P 131

In vitro *Mycobacterium tuberculosis* mutants resistant to bedaquiline and clofazimine display cross-resistance

Nabila Ismail[1], Remco Peters[2], Nazir Ismail[3], Shaheed Vally Omar[3]
[1]Medical Microbiology, University of Pretoria, South Africa
[2]University of Pretoria, Anova Health Institute, South Africa
[3]Centre for Tuberculosis, National Institute for Communicable Diseases, National Health Laboratory Services, Johannesburg, South Africa

Introduction: The phenomenon of cross-resistance can occur between two or more drugs due to mutation in a shared drug target. For bedaquiline, although the primary target is the ATP synthase enzyme (encoded by *atpE*) an additional target is reported to play a role, i.e. a repressor protein, *mmpR*, encoded by *rv0678*. This protein is responsible for repression of translation of the *mmpL5-mmpS5* efflux pump proteins, which influence drug entrance into the bacterium. It is mutation within this latter target (*rv0678*) that results in clofazimine cross-resistance. Additionally, clofazimine resistance is also marked by mutations in *rv1979c* and *rv2535*. Low level increases in bedaquiline MIC values have also been associated with *rv2535* mutation.
Materials/Methods: An adaptation of the Lüria- Delbruck assay was used for the isolation of spontaneous *Mycobacterium tuberculosis* mutants. Six clofazimine- and four bedaquiline-resistant mutants were isolated using fully susceptible and pyrazinamide-resistant ATCC strains. Mutants are confirmed phenotypically (MGIT960 DST) followed by performing clofazimine MIC determinations using bedaquiline-resistant mutants and *vice versa*. Phenotypic resistance was confirmed when MIC values were 2 µg/ml (proposed critical concentration is 1 µg/ml for both drugs). Validation was performed using whole genome sequencing through investigation of *rv0678*, *atpE*, *rv1979c* and *rv2535* genes.
Results: Four bedaquiline-resistant mutants with MIC values of 4->8 µg/ml and six clofazimine-resistant mutants with MIC values of 1-4 µg/ml were isolated from two ATCC strains. Three out of four bedaquiline-resistant mutants possessed *atpE* mutations detectable at a 10% frequency or higher. Of these, two did not display cross-resistance, while in the third, cross-resistance was exhibited and *rv0678* mutations identified (<10% frequency). The fourth bedaquiline-resistant mutant possessed an *rv0678*

mutation and displayed cross-resistance. All six clofazimine-resistant mutants possessing the *rv0678* mutation had MIC values of 4-8 µg/ml for bedaquiline.
Conclusion: We show that *in vitro* mutants without prior drug-exposure display cross-resistance between bedaquiline and clofazimine due to *rv0678* mutations. For a single mutant possessing only an *atpE* mutation, cross-resistance is displayed, possibly attributed to low frequency *rv0678* mutations-this requires further confirmation. These findings, particularly if clinically confirmed, warrant caution in designing TB drug regimens.

P 139

Emergence of bedaquiline resistance after completion of bedaquiline-based drug-resistant TB treatment: a case study from South Africa

Margaretha de Vos[1], Serej Ley[2], Brigitta Derendinger[1], Anzaan Dippenaar[3], Melanie Grobbelaar[4], Anja Reuter[5], Johnny Daniels[5], Scott Burns[6], James Posey[6], Grant Theron[1], Rob Warren[3], Helen Cox[8]
[1]DST/NRF Centre of Excellence for Biomedical Tuberculosis Research, Sa Mrc Centre for Tuberculosis Research; Division of Molecular Biology and Human Genetics, Faculty of Medicine and Health Sciences; Stellenbosch University, Cape Town, South Africa
[2]Stellenbosch University
[3]South African Medical Research Council Centre for Tuberculosis Research, DST/NRF Centre of Excellence for Biomedical Tuberculosis Research, Stellenbosch University, Cape Towm, South Africa
[4]NRF/DST Centre of Excellence for Biomedical Tuberculosis Research; SAMRC Centre for Tuberculosis Research, Stellenbosch University, Cape Towm, South Africa
[5]Medecins Sans Frontieres, South Africa
[6]Centers for Disease Control and Prevention, United States
[8]University of Cape Town, South Africa

Background: Treatment outcomes for drug-resistant tuberculosis (DR-TB) are poor with only 52% of MDR-TB and 24% of XDR-TB patients successfully treated. To address the global DR-TB epidemic WHO has released guidelines for the use of bedaquiline (BDQ) for the treatment of rifampicin-resistant or MDR-TB for specific indications. However, standardised methods to perform drug susceptibility testing (DST) have not been defined and BDQ resistance mechanisms remain poorly characterised.
Methods: Illumina NextSeq whole genome sequencing (WGS) was used to characterise serial *Mycobacterium tuberculosis (Mtb)* isolates from a patient receiving BDQ in Khayelitsha, South Africa. Phenotypic drug susceptibility testing (DST) for BDQ was performed in MGIT-960 media (concentration 1µg/ml).
Results: WGS showed an initial infection with a strain resistant to 7 drugs (rifampicin, isoniazid (low-level), ethambutol, ethionamide, fluoroquinolones, pyrazinamide and streptomycin). Following initial treatment failure with a standardised MDR-TB regimen, the patient was placed on a regimen containing 6 effective drugs (including BDQ, based on WGS). Isolates taken prior to BDQ initiation were BDQ-susceptible (phenotypically). WGS of subsequent serial isolates revealed the acquisition of a variant in R*v0678* (conferring BDQ-resistance) one month after stopping BDQ treatment. Subsequent isolates showed the loss and gain of several other *Rv0678* variants, with only one variant (138 G insertion) fixed in the last available isolate. All isolates with *Rv0678* variants were BDQ-resistant.
Conclusion: The systematic gain and loss of *Rv0678* variants in isolates taken after completion of BDQ-based treatment illustrates the complex ongoing evolution patterns of *M. tuberculosis* as the concentration of BDQ decreases in the patient (long half-life). An alternative explanation is the emergence of existing BDQ-resistant *Mtb* from lesions which rupture following continuation of treatment without BDQ and after stopping all TB treatment. The emergence of BDQ resistant *M. tuberculosis* following stopping of treatment poses a risk of transmission of BDQ resistant clones to close contacts. Monitoring of pre-existing and emerging BDQ resistance should be a priority for all routine use and should continue post BDQ cessation.

P 157

No evidence for cross-resistance between para-aminosalicylic acid and trimethoprim-sulfamethoxazole in *Mycobacterium tuberculosis*

Elena Martinez[1], Shaheed Vally Omar[2], Matthias Merker[3], Thomas Schön[4], Erik Sturegård[5], Danesh Moradigaravand[6], Julian Parkhill[6], Nazir Ismail[2], Vitali Sintchenko[7], Sharon Peacock[8], Stefan Niemann[3], Claudio Köser[9], Silke Feuerriegel[3]
[1]Centenary Institute and Marie Bashir Institute for Infectious Diseases and Biosecurity, University of Sydney, Australia
[2]Centre for Tuberculosis, National Institute for Communicable Diseases, National Health Laboratory Services, Johannesburg, South Africa

[3]***Division of Molecular and Experimental Mycobacteriology, Research Center Borstel, Germany***
[4]***Department of Clinical Microbiology and Infectious Diseases, Kalmar County Hospital, Sweden***
[5]***Region Skåne, Regional Center for Disease Control and Hospital Hygiene, Malmö, Sweden***
[6]***Wellcome Trust Sanger Institute, Hinxton, United Kingdom***
[7]***Centre for Infectious Diseases and Microbiology, The University of Sydney, Australia***
[8]***London School of Hygiene and Tropical Medicine, United Kingdom***
[9]***Department of Genetics, University of Cambridge, United Kingdom***

Contrary to earlier reports, *Mycobacterium tuberculosis* is not intrinsically resistant to trimethoprim-sulfamethoxazole (TMP/SMX), which is consequently increasingly being used to treat extensively drug resistant (XDR) tuberculosis. However, because TMP/SMX as well as *para*-aminosalicylic acid (PAS) target the folate metabolism, one or more mechanisms might confer cross-resistance to both drugs, limiting their clinical utility. In particular, the possibility was raised that TMP/SMX prophylaxis in HIV-positive patients may select for PAS cross-resistance in *M. tuberculosis*. To investigate the risk of cross-resistance, we determined PAS and TMP/SMX minimal inhibitory concentrations (MICs) using BACTEC 960 MGIT for the following strains of *M. tuberculosis*: i) a *thyA*-deletion mutant (H37RvΔ*thyA*) and the corresponding strain that was complemented with wild-type *thyA* (H37RvΔ*thyA* pASF63), (ii) clinical isolates from Australia that were strategically selected to cover all classical PAS resistance mechanisms (*thyA*, *dfrA*, *Rv2671* and *folC*), (iii) PAS and TMP-SMX susceptible control isolates from Australia and Sweden. Moreover, we analysed binary PAS drug-susceptibility (DST) results for otherwise pan-susceptible isolates from a high HIV-setting (Cape Town, South Africa). H37RvΔ*thyA* was fully resistant to PAS (>2048 mg/L), whereas the susceptible phenotype could be restored in H37RvΔ*thyA* pASF63 (2 mg/L). The TMP/SMX MIC for both strains as well as the H37Rv parent were identical (1.024/19.2 mg/L). PAS MICs for clinical isolates with known PAS resistance mutations varied between 8 and 2048 mg/L, depending on the underlying mechanism, compared with ≤2 mg/L for susceptible isolates. By contrast, the MICs for TMP/SMX for all clinical isolates and H37Rv ATCC 27294 showed relatively little variation (0.256/4.8-2.048/38.4 mg/L). Moreover, no PAS mono-resistance in otherwise pan-susceptible isolates from Cape Town was observed following repeated DST to eliminate random false-resistant PAS results. In conclusion, we did not observe any evidence of cross-resistance between PAS and TMP/SMX due to four known drug resistance mechanisms, although a better understanding of the MIC distribution of phenotypically wild-type strains is essential to define epidemiological cut-off values (ECOFFs) for both drugs. Pending more clinical efficacy and pharmacokinetic/pharmacodynamic (PK/PD) data, PAS and TMP/SMX can thus be regarded as distinct antimycobacterial agents, despite targeting the same pathway.

P 176

Isoniazid resistance in *Mycobacterium tuberculosis* is a heterogeneous phenotype comprised of overlapping MIC distribution with different underlying resistance mechanisms

Arash Ghodousi[1], Emanuele Borroni[1], Claudio U. Köser[2], Daniela Maria Cirillo[1]
[1]***Emerging Bacterial Pathogens Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy***
[2]***Department of Genetics, University of Cambridge, United Kingdom; Molecular and Experimental Mycobacteriology, Research Centre Borstel, Germany***

The endorsements by WHO of the short-course MDR TB regimen and, more recently, of novel treatment recommendations for isoniazid (INH) mono-resistant TB, have triggered a debate about whether and under which circumstances the benefits of using INH at either the standard or higher dose to treat strains that are resistant to this drug outweigh its side-effects.
To advance this discussion, we used the BACTEC 960 MGIT system to conduct comprehensive minimum inhibitory concentration (MIC) testing with two-fold dilution series for 51 INH resistant strains. The resulting MIC distributions were correlated with mutations in the classical INH-resistance genes (i.e. *katG*, *inhA*, *fabG1*, and *ahpC*) using whole-genome sequencing.
We observed four populations, which corresponded to distinct underlying resistance mechanisms (or combinations thereof) and correlated with increasing levels of INH resistance: strains with i) only *inhA* promoter mutations, ii) only *inhA* promoter mutations with a concurrent *inhA* coding mutation, iii) only *katG* S315T/N mutations, or) *katG* S315T/N mutations that also harboured an *inhA* promoter mutation.
Notably, even though the modes of these MICs distributions were distinct, the distributions as a whole did overlap to some extent. This means that if is impossible to define breakpoints that reliably distinguish these mechanisms using

phenotypic methods. Instead, “expert rules” are likely needed that combine phenotypic and genotypic results to resolve this conundrum.

P 197

Investigation of in vitro Antituberculosis Activity in the Plant Extracts from St. John´s wort (Hypericum perforatum L.) and Aloe Vera

Efdal Oktay Gültekin[1], Mahmut Ülger[2], Erdal Yabalak[3], Nurbanu Kurnaz[1], Seda Tezcan Ülger[1], Nuran Delíalíoğlu[1], Ahmet Murat Gizir[3], Gönül Aslan[1]
[1]Faculty of Medicine, Department of Medical Microbiology, Mersin University, Turkey
[2]Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Mersin University, Turkey
[3]Faculty of Arts and Science, Department of Chemistry, Mersin University, Turkey

Objectives: Tuberculosis (TB) is a highly infectious disease declared a global health emergency by the World Health Organization, with approximately one third of the world's population being latently infected with *Mycobacterium tuberculosis*. High incidence of infection and the increased rate of multi-drug resistant and extensively-drug resistant isolates of the organism further complicated the problem of TB control and have called for an urgent need to develop new anti-TB drugs from plants. St. John□s wort (*Hypericum perforatum L*.) and aloe vera plant extracts have been using in the treatment of various diseases in our country's traditional. In this study, it was aimed to investigate the *in vitro* activity of the plant extracts from St. John□s wort (*Hypericum perforatum L.*) and aloe vera against *Mycobacterium tuberculosis* H37Rv strain.
Methods: Methanolic extracts of the plant extraction were obtained by Soxhlet method. The antimycobacterial activity was determined using 96 wells of microplate with the help of visual Resazurin Microtiter Assay. Minimum inhibitory concentrations (MICs) of plants were determined against *Mycobacterium tubercluosis* H37Rv at concentrations of 250-1.9 µg/mL. Rifampin (RIF) and isoniazid (INH) were used as standard drugs.
Results: MIC values of St. John□s wort and aloe vera extracts and methanol (solvent of plants) were evaluated as 31.25 µg/ml, 62.5 µg/ml, 31.25 µg/ml for *Mycobacterium tuberculosis* H37Rv respectively. The MIC values of both RIF and INH against *Mycobacterium tubercluosis* H37Rv were 0.03 µg/ml.
Conclusion: According to our result plant extracts showed low antituberculosis activity against to standard drugs. It is believed that these plants may not have therapeutic value in the treatment of TB. However, further investigations are needed on isolating chemical constituents responsible for eliciting the observed activity in these plants.
Keywords: *Mycobacterium tuberculosis,* Tuberculosis, Antituberculosis activity, *Hypericum perforatum L.*, Aloe vera

P 211

Xanthates: metabolsim by flavoprotein-containing monooxygenases and antimycobacterial activity

Violeta Valcheva[1], Tsveta Stoyanova[2], Paul R. Ortiz de Mantellano[3], Stanislav Yanev[2]
[1]Department of Infectious Microbiology, Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria
[2]Department of Toxycology, Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria
[3]Department of Pharmaceutical Chemistry, University of California, United States

Background: Ethionamide (ETH) plays a central role in the treatment of tuberculosis in patients resistant to the first-line drugs. The ETH, thioamide, and thiourea class of antituberculosis agents are pro-drugs that are oxidatively converted to their active S-oxides by the flavin monooxygenase EtaA of *Mycobacterium tuberculosis*, thus initiating the chain of reactions that result in inhibition of mycolic acid biosynthesis and cell lysis.
Here we present results from the study of metabolism of several xanthates by EtaA and FMO3 to S-oxygenated metabolites, and compare the nature and reactivity of the resulting metabolites with those from ETH.
Methods: Potassium salts of various xanthates were synthesized as previously described by Rao (1971). The metabolism of various xanthates was studied spectrophotometrically. The in vitro antimycobacterial activity of C2, C5, C8, D609 and ETH was determined through the proportional method of Canetti (Canetti et al., 1969) towards reference strain M. tuberculosis H37Rv.
Results: The xanthates with short to medium alkyl chain substituents had MIC values ten times lower than that of ETH, whereas the MIC value of D609, compound with a cyclic substituent, was comparable to that of ETH. As a whole, some of the xanthate analogues tested in this study showed a moderate level of bacteriostatc antimycobacterial activity, in most cases comparable or even higher than

that of the reference drug ethambutol Several xanthates are oxidized by purified EtaA to S-oxide metabolites (perxanthates) implicated in their antimycobacterial activity. This process is analogous to that responsible for activation of ETH. EtaA was not inhibited in a time-dependent manner during the reaction. Xanthates with longer alkyl chain were oxidized more efficiently. EtaA oxidized octyl-xanthate (Km = 5 μM; Vmax = 1.023 nmolP/min; kcat = 5.2 molP/min/mole) more efficiently than ETH (194 μM; 1.46 nmolP/min; 7.73 nmolP/min/mole, respectively). Furthermore, the in vitro antimycobacterial activity of four xanthates against M. tuberculosis H37Hv was higher (MIC around 1 μM) than that of ETH (12 μM).

Conclusions: The examined xanthates exhibited high affinity for FMOs that oxidized them to the corresponding S-oxides (perxanthates), apparently without altering the enzyme activity. They could be used as test substrates for rapid and selective determination of FMO activity in various biological media. Xanthates have antimycobacterial activity against M. tuberculosis H37Rv comparable to that of ETH. The synthesis of further xanthate derivatives could present a promising route to compounds with higher antimycobacterial activity.

P 5

Mycobacterial community structure in the soil environments determined with hsp65 targeted amplicon sequencing

Tomoaki Ichijo[1], Daisuke Shimizu[1], Nobuyasu Yamaguchi[2], Naoki Kagimura[1], Masao Nasu[3]
[1]Osaka University, Suita, Osaka, Japan
[2]Osaka Institute of Public Health, Osaka, Japan
[3]Osaka Ohtani University, Tondabayashi, Osaka, Japan

The number of patients with nontuberculous mycobacterial diseases has been increasing all over the world since the 1990's, especially in developed countries. Although it is thought that aerosol generated in the surrounding aquatic environment and soil environment is the vehicle of the bacteria, the infection source and the infection route remains unclear. In order to prevent the disease originated from environments, clarification of the dynamics of nontuberculous mycobacteria in the environment is indispensable. Therefore, in this study, we focused on the soil considered as one of infectious sources, and clarified the mycobacterial abundance and community structure using gene-targeting methods. Surface soil at 23 points in Osaka University (3 gardens, 10 under trees, 6 sites with vegetation, 4 plants with no vegetation) and 4 plant pot soils were taken and microbial DNA in soil samples was extracted. The bacterial and mycobacterial abundance was determined with quantitative PCR method targeting 16S rRNA gene and *atpE* gene, respectively. In addition, mycobacterial community structures in environments were determined with the *hsp65* gene targeted amplicon sequencing. Bacteria and mycobacterial abundances in soil samples were measured and found to be 10^7-10^9 cells/g and 10^5-10^7 cells/g, respectively. In the samples, except for the dried samples, it was found that mycobacteria were present in a ratio of 0.1 to 10% based on the total bacteria. Analysis of about 2,000 reads generated from amplicon sequencing targeting *hsp65* gene revealed that there were various species of nontuberculous mycobacteria. Among these, focusing on *Mycobacterium avium,* clinically important species in Japan, it was found that it existed widely in the soil analyzed in this study, and it might dominate in the environment that repeats drying and wetting like flower pots. These results became the fundamental knowledge for understanding the dynamics of nontuberculous mycobacteria in the environment, and we can contribute to prevent the nontuberculous mycobacterial diseases from the viewpoint of environmental microbiology.

P 22

Genomic comparison of pathogenic and non-pathogenic mycobacteria using *Mycobacterium bovis* Bacillus Calmette-Guérin as a model

Maria Carolina Sisco Zerpa[1], Marlei Gomes[1], Luciana Distásio de Carvalho[2], Carlos Eduardo Dias Campos[2], Paulo Cesar de Souza Caldas[2], Jesus Pais Ramos[2], Beatriz Lopez[3], Leila Mendonça-Lima[4], Jacobus De Waard[5], Rafael Silva Duarte[1], Philip Noel Suffys[6]
[1]Laboratório de Micobactérias, Universidade Federal Do Rio de Janeiro, Brazil
[2]Centro de Referência Professor Hélio Fraga, Rio de Janeiro, Brazil
[3]Instituto Nacional de Enfermedades Infecciosas, Buenos Aires, Argentina
[4]Laboratório de Genômica Funcional e Bioinformática, Instituto Oswaldo Cruz, Fiocruz, Rio de Janeiro, Brazil
[5]Laboratório de Tuberculosis, Servicio Autónomo Instituto de Biomedicina Dr. Jacinto Convit, Caracas, Venezuela
[6]Laboratório de Biologia Molecular Aplicada Às Micobactérias. Instituto Oswaldo Cruz, Fiocruz, Rio de Janeiro, Brazil

Mycobacterium bovis Bacillus Calmette-Guérin (BCG), the vaccine against tuberculosis, is derived from the pathogenic *Mycobacterium bovis*. The deletion of the Region of Difference 1 (RD1) is one of the causes of the loss of its virulence. Although considered safe, complications after vaccination may occur, such as local or disseminated infection, named BCGitis. The mechanisms that control the conversion of a non-virulent microorganism to a pathogenic one in humans are not entirely understood. Genome modifications like single nucleotide polymorphisms (SNP), duplications, insertions and deletions could be responsible for returning to a virulent state. By collecting clinical isolates from patients with BCGitis and comparing their genomes to those of the

vaccine strains available at NCBI, we aimed to characterize the genetics modifications responsible for the possible conversion of the non-virulent vaccine into a pathogen. We have analyzed 26 BCG clinical isolates from patients vaccinated in Brazil, Venezuela and Argentina. The strains were sequenced using the Illumina Hiseq 2500 platform and the Nextera® XT DNA Sample Preparation kit. Upon variant call analysis using the Bionumerics software three different SNPs were detected among three isolates from Brazil, two SNPs belonged to the same strain, one synonymous SNP (sSNP) located in the 3-oxoacyl-[acyl-carrier protein] reductase gene and a non synonymous SNP (nsSNP) in the DNA-directed RNA polymerase beta subunit gene, both being transitions; a second strain presented a nsSNP transition in a gene coding for a hypothetical protein. Among the six isolates from Venezuela, five different SNPs were observed in a single strain, a nsSNP transition in the macrolide ABC transporter ATP-binding protein, a nsSNP transversion in the Nudix-related transcriptional regulator NrtR gene, a sSNP transition in the Formamidopyrimidine-DNA glycosylase gene, a nsSNP transversion in the F420-0--gamma glutamyl ligase gene and one SNP in an intergenic region. One strain had a sSNP transition on an intergenic region while another strain presented a sSNP transversion in a gene coding for a hypothetical protein. Among the 17 isolates from Argentina two strains harbored nsSNP transitions, one in the type VII secretion protein EsxD and the other on a PE family protein. The SNP analysis was also performed using Snippy software. The vaccine strains will be resequenced and also a larger number of clinical strains from the three countries will be added to the study. Other analytical approaches such as detection of complex SNPs, gene deletions, duplications and insertions are underway. Our results demonstrate evidence of mutations occurring in the genome of clinical BCG strains, this could be associated to changes in the mechanisms of bacterial pathogenesis and the conversion of the non-virulent vaccine into a virulent microorganism.

P 51

Non-Tuberculous Mycobacterial healthcare-associated infections, a French 3-years retrospective study

Emmanuel Lecorche[1], Daniau Côme[2], Faïza Mougari[1], Hanaa Benmansour[1], Jérôme Robert[3], Anne Berger-Carbonne[2], Emmanuelle Cambau[4]

[1]National Reference Center for Mycobacteria and Antimycobacterial Resistance (CNR-MYRMA); APHP, Lariboisère Hospital, Microbiology Department, University Paris Diderot, France

[2]Santé Publique, France

[3]Centre National de Référence des Mycobactéries et de la Résistance des Mycobactéries Aux Antituberculeux (CNR-MYRMA), Bactériologie-Hygiène, Hôpitaux Universitaires Pitié Salpêtrière-Charles Foix, France

[4]Hôpital Lariboisière; National Reference Center for Mycobacteria and Antimycobacterial Resistance (CNR-MYRMA); Service de Bactériologie-Virologie, France

Non-tuberculous mycobacteria (NTM) are ubiquitous bacteria that can be found in soil, water and in human-engineered environment. NTM infections usually occur in immunocompromised patients but also in immunocompetent patients following invasive procedures. Such healthcare-associated infections are an increasing problem for health care providers and policy makers. We conducted a retrospective study of NTM healthcare- associated infections in France between January 2012 and March 2017, using databases of the French National Public Health Agency (Santé Publique France) or the National Reference Center for Mycobacteria and Antimycobacterial Resistance.

NTM healthcare-associated infection cases were identified in 47 patients from 24 distinct centers. NTM cases were due to 9 mycobacteria species: M. fortuitum complex (n = 15 cases), M. chelonae (n = 14), M. abscessus (n = 9), M. chimaera (n = 3), M. marinum (n = 2), M. neoaurum (n = 1), M. wolinskyi (n = 1), M. lentiflavum (n = 1) and M. fuerthensis (n = 1) and no identification was found in 2 cases (acid-fast bacilli positive and culture negative). Cases distributed as 33 related to surgical procedures (cardiac surgery n = 12 cases, breast implant reconstruction n = 11, orthopedic surgery n = 7 and skin surgery n = 3), 9 to invasive procedures (digestive endoscopy n = 2, catheter infection n = 2, mesotherapy n = 2, tattoo n = 2 and joint infiltration n = 1), 2 to non-invasive care (pool therapy n = 2) and no causes were found in 2 cases.

Environmental investigations have been performed in 14 cases, water was confirmed as the source of contamination in 3 cases.

Medical devises or prosthesis were suspected for 6 cases. Corrective measures concerning hygiene practices and control of environment were described in 9 cases.

Healthcare-associated NTM infections are rare but these cases stress the need of notifications in outpatient settings, investigations with mycobacterial-specific diagnostics and both medical and environmental health expertise. A better knowledge of the epidemiology of healthcare-associated NTM infections will improve effective prevention strategies.

P 63

Relative risk of all-cause mortality in patients with nontuberculous mycobacterial lung disease in US managed care

Theodore Marras[1], Christopher Vinnard[2], Keith Hamilton[3], Gina Eagle[4], Engels Chou[4], Raymond Zhang[4], Marko Obradovic[5], Roald van der Laan[5], Quanwu Zhang[4]
[1]***University Health Network Toronto, Canada***
[2]***New Jersey Medical School - Rutgers, The State University of New Jersey, United States***
[3]***University of Pennsylvania, Philadelphia, United States***
[4]***Insmed Inc., Bridgewater, United States***
[5]***Insmed Europe, Huijbergen, The Netherlands***

Purpose: A health insurance claims database study was conducted to evaluate the relative risk of all-cause mortality and risk factors between patients diagnosed with nontuberculous mycobacterial lung disease (NTMLD) and an age-sex–insurance coverage matched control group.
Methods: Patients from a national managed care insurance plan with physician claims for NTMLD on ≥2 separate occasions ≥30 days apart (n=2005) were identified between 2007 and 2016. A control group without NTMLD (n=6014) was matched 3:1 to the NTMLD sample according to age, sex and insurance coverage period. The date of first NTMLD diagnosis was assigned to the matched controls as an index date. All individuals in the analysis had ≥12 months of healthcare coverage before the index date. Mortality data originated from the Social Security Death Master File. The number of mortality records after 2011 was reduced about 30% owing to local reporting decisions. A Cox regression was used to compare survival between NTMLD patients and controls, adjusting for demographic factors and baseline health indicators.
Results: Mean age was 67 years, and 66% were female. Mean follow-up time to death or to data cutoff date was 3.4 years for NTMLD and 3.65 years for controls. Mean Charlson comorbidity index score was 2.2 in NTMLD patients vs 0.5 in controls. Selected baseline health conditions in NTMLD vs controls included 36.5% vs 0.1% bronchiectasis, 52.0% vs 5.9% COPD, 44.5% vs 1.7% pneumonia, 23% vs 3.5% asthma, 2.1% vs 0% cystic fibrosis, 15.4% vs 12.4% diabetes, 3.8% vs 4.0% obesity, 9.9% vs 3.8% depression, 6.1% vs 0.6% lung cancer, 55.1% vs 14.6% use of immunosuppressive drugs, 23.0% vs 4.0% tobacco use, 2% vs 0.1% HIV infection, and 1.6% vs 0.1% organ transplant. The rate of all-cause mortality from the index date was 20.7 in NTMLD patients vs 5.6 in controls per 1000 person-years (rate ratio=3.73; 95% CI: 2.93-4.75). Multivariable adjusted Cox regression showed a doubling risk of all-cause mortality (hazard ratio [HR]=2.11; CI: 1.56-2.85; *P*<.001) in the NTMLD vs control group. The Cox model also showed that mortality more than doubled (HR=2.35) with each additional 20 years of age (*P*<.001) and increased 48% with male sex, 540% with moderate or severe liver disease (*P*<.001), 87% with lung cancer (*P*=.01), 65% with a psychiatric disorder (*P*=.002), 100% with an immune deficiency (*P*=.006), and 44% with use of an inhaled corticosteroid agent (*P*=.02). Obesity (HR=.31; *P*=0.02) and atherosclerosis (HR=.57; *P*=.035) were associated with a decreased risk of all-cause mortality.
Conclusions: All-cause mortality more than doubled with NTMLD compared with an age-sex–insurance coverage matched control group in a large US national managed care insurance plan, even after adjustment for other risk factors. The incremental risk of all-cause mortality in NTMLD compared to controls represents a critical unmet medical need and requires effective management of the disease.

P 64

Population-based incidence and prevalence of nontuberculous mycobacterial lung disease in a large US managed care health plan, 2008-2015

Kevin Winthrop[1], Gina Eagle[2], Roald van der Laan[3], Marko Obradovic[3], Raymond Zhang[2], Quanwu Zhang[2]
[1]***Oregon Health & Science University, Portland, United States***
[2]***Insmed Inc., Bridgewater, United States***
[3]***Insmed Europe, Huijbergen, The Netherlands***

Rationale: Despite increasing recognition of the public health impact of nontuberculous mycobacterial lung disease (NTMLD), population-based reports on incidence and prevalence of NTMLD are only sparsely available in the United States. This study estimated the yearly NTMLD incidence and prevalence between 2008 and 2015 in a large US managed care claims database.
Methods: A cohort of 16,872 insured members, diagnosed for NTMLD with an ICD9 031.0 or ICD10 A31.0, was identified from a US national health insurance plan. Each Individual who was diagnosed with NTMLD on at least two separate occasions ≥30 days apart was considered as a positive NTMLD identification, yielding 9,476 positively identified patients. For incidence,

an NTMLD diagnosis in a calendar year was considered as a new case by ascertaining absence of the diagnosis during the prior year and 24-month continuous medical insurance coverage. For prevalence, all NTMLD diagnoses in a year were considered as prevalent cases and continuous medical insurance coverage of the year was required.

Results: People ≥60 years of age at the first available NTMLD diagnosis comprised 69.4% of the patient cohort. The overall NTMLD incidence increased from 3.13 in 2008 to 4.73 in 2015 per 100,000 plan members (Table 1). People <65 years had an incidence of 1.34 in 2008 and 1.82 in 2015 per 100,000 members. People ≥65 years had an incidence of 12.70 in 2008 and 18.37 in 2015 per 100,000 members. The increase in NTMLD incidence from 2008 to 2015 was 35.8% in people <65 years compared to 44.6% in people ≥65 years. Incidence was 2.05 in 2008 vs 2.71 in 2015 per 100,000 men, compared to 4.16 in 2008 vs 6.69 in 2015 per 100,000 women. The overall prevalence increased from 6.64 in 2008 to 11.72 in 2015 per 100,000 plan members. The respective yearly prevalence rates were 2.87 in 2008 and 4.10 in 2015 per 100,000 people <65 years vs 30.27 and 47.48 per 100,000 people ≥65 years, and 3.79 in 2008 and 6.45 in 2015 per 100,000 men vs 9.63 and 16.78 per 100,000 women.

Conclusions: This study provides the most recent NTMLD epidemiological data from the claims database of a large US managed care health insurance plan. While NTMLD is rare in the US population, the public health impact of increasing NTMLD incidence and prevalence is important to consider in health policy and health care decision making.

P 114

Species Distribution of Nontuberculosis Mycobacteria Isolated from Pulmonary and Extrapulmonary Samples in Turkey (2017)

Hulya Simsek[1], Ahmet Arslantürk[1], Meryem Demir[1], Derya Altun[1], Alper Sarıbaş[1], Nilay Uçarman[1]

[1]Ministry of Health, General Directorate of Public Health, National Tuberculosis Reference Laboratory, Ankara, Turkey

Aim: In the recent years, incidence of Nontuberculous Mycobacteria (NTM) infections has been increased gradually in the world. Thus NTM have become more important in terms of public health as opportunistic disease-causing agents in immunocompromised patients. They can cause several diseases with significant morbidity and mortality. There has not yet any comprehensive study representing distribution of NTM species covering all regions of Turkey. The aim of this study was to show distribution of NTM species isolated from pulmonary and extrapulmonary samples in National Tuberculosis Reference Laboratory.

Method: The samples used in this study were collected from various regions in Turkey between 01 January and 31 December 2017. 414 clinical isolates growth on LJ slants or MGIT 960 liquid media were evaluated for identification of NTM after differentiating of M. tuberculosis complex and NTM by rapid immunochromatograpic test. It was used GenoType Mycobacterium CM/AS assay (Hain Lifescience) to differentiate NTM from *Mycobacterium tuberculosis* complex (MTBC) members.

Results: Of 414 NTM species**,** 95.7% were isolated from pulmonary (sputum, BAL, bronchial lavage, pleural fluid and tracheal aspirate), while 4.3% were extrapulmonary (tissue biopsy, urine, gastric lavage and abscess) samples. Distribution of the identified strains was *M. abscessus* complex (62), *M. avium* (22), *M. chelonae* (25), *M. chimeara* (3), *M. fortuitum* group (59), *M. gordonae* (62), *M. heckeshornense* (1), *M. intracellulare* (40), *M. kansasii* (32), *M. lentiflavum* (24), *M. mucogenicum* (7), *M. simiae* (9), *M. szulgai* (10), and *M. xenopi* (3). 55 NTM species could not be identified by the method that we used.

Conclusion: In 2017, 14 different NTM were identified from pulmonary and extrapulmonary samples. But the number of species would increase, when 55 NTM species are identified by other methods. Distribution of NTM was similar to that of our previous study, although incidence of NTM has been increased in our country. *M. heckeshornense* was a new species identified in our laboratory.

Key words: Nontuberculous Mycobacteria, pulmonary samples, extrapulmonary samples, GenoType Mycobacterium CM/AS kit.

P 130

Characterization of virulence and genome analysis of *Mycobacterium kansasii* strains isolated from patients ith pulmonary disease in Brazil

Philip Noel Suffys[1], Vinicius Mussi[2], Fabricio Almeida[2], Sanderson Calixto[2], Thatiana Ventura Simão[2], Luciana Distacio[3], Edson Machado[4], Marcos Catanho[4], Antonio Basilio de Miranda[5], Elena Lasunskaia[2]

[1]Laboratory of Molecular Biology Applied to Mycobacteria, Oswaldo Cruz Institute, Fiocruz, Rio de Janeiro, Brazil; Insitute of Tropical Medicine in Antwerp, Belgium

[2]*Laboratory of Biology of Recognition, UENF, Campos dos Goytacazes, Rio de Janeiro, Brazil*
[3]*Centro de Referencia Prof. Helio Fraga, Curicica, Rio de Janeiro, Brazil*
[4]*Laboratory for Functional Genomics and Bioinformatics, Oswaldo Cruz Institute, Fiocruz (IOC), Rio de Janeiro, Brazil*
[5]*Laboratory of Computational and Systems Biology, IOC, Rio de Janeiro, Brazil*

Introduction: Pulmonary disease due to nontuberculous mycobacteria (NTM) is increasing worldwide and in the state of Rio de Janeiro in Brazil, a higher prevalence of *M. kansasii* (*Mkan*) is observed. In this study, we evaluated virulence of *Mkan* strains isolated in Brazil from patients with pulmonary disease in comparison with the virulence of the reference *Mkan*ATCC 12478 strain.
Methods and results: *Mkan* strains were isolated in Rio de Janeiro (n=7), Pernambuco (n=3), Rio Grande do Sul (n=1) and Santa Catarina (n=1). Screening for virulence was based on *in vitro* evaluation of bacterial ability to intracellular growth and induction of necrotic death in murine RAW264.7 macrophages and production of TNF-a, IL-1b and IL-10 in culture supernatants. Strain virulence could be divided into three groups with high, intermediate and low virulence. The strains from the highly virulent group that produced particularly high levels of TNF and no IL-1 and IL-10 were suggested to display increased virulence *in vivo,* different from the less virulent strains, assayed by intratracheal high dose-infection of C57BL/6 mice. The most virulent strain 8835 killed mice within 40 days post-infection while mice infected with other strains maintained viability up to the end of observation. In addition, strains differed in the induction of severity of lung lesions, including inflammation leading to intragranulomatose necrosis formation of solid granulomas. In parallel, in search of the genetic basis that determines the pathogenicity of *Mkan* isolates is being studied on basis of whole genomes of these strains and comparative genomics to evaluate genetic polymorphisms in putative mycobacterial virulence genes.
Conclusion: Considerable difference in virulence of *Mkan* clinical isolates was observed in the *in vitro* study based on infection of macrophage culture. The predicted virulence level was confirmed by the results obtained in animal model of infection and for the first time, an *Mkan* isolate lethal for C57BL/6 mice was observed. The feasibility to predict the virulence of *Mkan* isolate from the genome sequence is under investigation.
Financial support: FAPERJ, CNPq

P 143

Functional analysis of essential mycobacterial cell division genes

Susanne Gola[1]
[1]*National Center for Biotechnology CNB-CSIC, Madrid, Spain*

Current research increasingly points to unusual mechanisms of mycobacterial growth and cell division as the cause of intrinsically heterogeneous cellular populations whose single members can differ e.g. in their susceptibility to antibiotics.
We investigate the essential components of the mycobacterial divisome, a multi-protein complex that assembles in a ring-like structure in order to divide the cell. Divisome assembly is coordinated in space and in time. Early division proteins form, together with FtsZ, the proto-ring that is attached to the cytoplasmic membrane and serves as a scaffold for the recruitment of downstream protein. The late divisome components connect the proto-ring to the peptidoglycan synthesis machinery.
Mycobacteria rely on a reduced set of core divisome proteins and our results show that the essential SepF is the main early FtsZ-accessory protein of the mycobacterial proto-ring. Analysis of a *Mycobacterium smegmatis* FtsQ depletion strain indicates that this protein is a late mycobacterial divisome component, because the assembly of SepF is independent of the presence of FtsQ. Depriving cells of either the early or the late divisome proteins impedes proliferation and leads ultimately to cell death, which suggests that the mycobacterial division machinery could serve as a target structure for new drugs.

P 164

Prevalence of aminoglycoside and macrolide resistance among Slovenian M. abscessus and M. intracelullare isolates

Sara Truden[1], Manca Zolnir-Dovc[1], Nadja Polanc[1]
[1]*Laboratory for Mycobacteria, University Clinic of Respiratory and Allergic Diseases Golnik, Slovenia*

Background: *Mycobacterium (M.) abscessus* and *M. intracellulare* belong to the nontuberculous mycobacteria (NTM). Due to their natural habitat, NTM are constantly exposed to various concentrations of antimicrobial drugs and other chemicals and consequently they had

developed different mechanisms of resistance: natural or acquired mechanisms. Macrolides and aminoglycosides are frequently used drugs to treat infections due to *M. abscessus* complex (MABSC) and *M. avium* complex (MAC). Important improvement was finding molecular mechanisms that involve detection of mutations in three resistance related genes: *erm*(41), *rrl* and *rrs*. Genes *erm*(41) and *rrl* are both responsible for macrolide resistance, meanwhile *rrs* gives an aminoglycoside resistance. Gene *erm*(41) is present only in MABSC meanwhile *rrl* and *rrs* can be detected in both groups of mycobacteria. Aim of our study was to present how many isolates have inducible macrolide resistance and how many isolates have aminoglycoside resistance.

Methods: Two hundred and eight Slovenian isolates were obtained from January 2000 to December 2017 from Slovenian National NTM Collection Golnik that belong to MAC and MABSC were analyzed. Molecular test GenoType NTM-DR (Hain Lifescience, Nehren, Germany) was used to detect genes *erm*(41), *rrl* and *rrs.*

Results: In total of 38 isolates of *M. abscessus* and 170 isolates of *M. intracellulare* were analysed. Among *M. intracellulare* isolates macrolide or aminoglycoside resistance was not detected. On the other hand, 38 isolates of *M. abscessus* were analysed and 31 had a macrolide resistance due to mutation in *erm*(41) gene. Six isolates belonged to *M. abscessus* subsp. bolletii and 25 belonged to *M. abscessus* subsp. *abscessus.* Macrolide resistance due to mutation in gene *rrl* was not detected. None of our *M. abscessus* isolates had an aminoglycoside resistance.

Conclusion: Our nation-wide analysis has showed that none of Slovenian *M. intracellulare* isolates has macrolide or aminoglycoside resistance. Furthermore, aminoglycoside resistance in *M. abscessus* isolates was not detected. Mutation in *erm*(41) gene was detected in all (n=6) *M. abscessus* subsp. *bolletii* isolates and in 80% of *M. abscessus* subsp. *abscessus* that leads to inducible macrolide resistance but mutation in *rrl* gene was absent. We can conclude that mutation in *erm*(41) is frequently detected in Slovenian *M. abscessus* isolates and prevalence of inducible macrolide resistance is considerably high.

Use of Multilocus Seuqnce Typing of *Mycobacterium xenopi* to distinguish relapse from reinfection in a pulmonary patient

María Teresa Tórtola Fernández[1], Carlos Escartin[1], Miguel Fernández Huerta[1], Maria Carmen Vivas[1], Josep Vegue[1], Monica Mena[1]
[1]***Micorbiologia, Hospital Vall D'hebron, Barcelona, Spain***

Introducción: *Mycobacterium xenopi* is an opportunistic pathogen; it affects people with previous lung diseases and immunosuppressed patients.
Our objective has been to evaluate the MSLT technique for *M.xenopi* to distinguish between relapse and reinfection.
Material and methods: ***Bacterial strains***. Two isolates of *M.xenopi* obtained in 2015 and 2017 from two sputum from the same patient with chronic obstructive pulmonary disease (COPD).
DNA extraction. DNA was extracted using a modified boiling lysis method using InstaGene Matrix (Bio-Rad). ***PCR.*** We used **s**ix genes (*atpD*, *fusA1*, *glnA1*, *pheT*, *secA1*, and *topA*) for MLST of *M. xenopi* described by Alexander et al (1). Microchip electrophoresis System for DNA/RNA Analysis (MultiNA, Shimazu) was used to confirm amplification of each MLST target and estimate amplicon concentration.
DNA sequencing. The BigDye Terminator v3.1 cycle sequencing kit (Life Technologies Inc.) was used for DNA sequencing of amplicons for MLST. Editing, analysis, and alignment of DNA sequences were performed with MEGA 5.2.
Results: For the interpretation of the results we use the allelic variants described by Alexander et al (1). DNA sequencing results for the MLST targets are summarized in Table 1 and 2.
Table1. MLST analysis of *Mycobacterium xenopi*

	N. of variants						
MLST-6ST	***atpD***	***fusA1***	***glnA1***	***pheT***	***secA1***	***topA***	**No. of isolates**
ST-1	1	1	1	1	1	1	0
ST-2	1	1	1	1	2	1	2

ST-1 The MLST profile is the allelic sequence of *M.xenopi* Type strain RIVM700367 obtained from BLAST (https://blast.ncbi.nlm.nih.gov/)

Table 2. MLST-6 analysis of consecutive isolates from individual patients

Patient	**Isolate**	**Date (mo/yr)**	**MSL-6ST**
1	1	11/2015	ST-2
	2	7/2017	ST-2

The two *M. xenopi* isolates obtained from the same patient showed an identical ST and was ST-2. Individual genes (*atpD*, *fusA1, topA*, *glnA1* and *pheT*) were found not to have allelic variants, only *secA1* gen have one allelic variant that was described by Alexander et al. This allelic variant was a synonymous SNPs ($G_{412} \rightarrow A$).
Conclusions: In the patient studied the two isolations of *M.xenopi* had an identical ST which means a relapse or that *M.xenopi* has persisted for two years in his lungs since in this type of patients it is necessary to differentiate between colonization and infection, for this purpose they must use the American Thoracic Society criteria. The MLST is a useful technique to distinguish between relapse and reinfection in a clinical diagnostic laboratory.
1.Alexander DC, Marras TK, Ma JH, Mirza S, Liu D, Kus JV, Soualhine H, Escuyer V, Warshauer D, Brode SK, Farrell DJ, Jamieson FB. Multilocus sequence typing of Mycobacterium xenopi. J Clin Microbiol. 2014 Nov;52(11):3973-7.

P 226

Differential drug susceptibility patterns of mycobacterium chimaera and other members of the *mycobacterium avium* complex

Florian P. Maurer[1], Philipp Pohle[1], Margit Kernbach[1], Daniela Sievert[1], Michael Hombach[2], Doris Hillemann[1], Katharina Kranzer[1]
[1]***National Reference Center for Mycobacteria, Borstel, Germany***
[2]***Roche Diagnostics International AG, Rotkreuz, Switzerland***

Introduction: The epidemiological cutoff (ECOFF) value, has been introduced by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) as a basis for standardized development of clinical breakpoints (CBPs). ECOFF values can provide orientation for informed therapeutic decision making in the absence of or amending CBPs. Comparison of the minimal inhibitory concentration (MIC) value of a particular clinical isolate with the respective ECOFF allows to assess the presence of acquired resistance mechanisms leading to a categorization of the clinical isolate as “wild-type” or “non wild-type”.
The slow-growing *Mycobacterium chimaera* is increasingly recognized as emerging nosocomial pathogen, but little is known regarding the drug susceptibility patterns of clinical *M. chimaera* isolates. To our knowledge, there are no published studies investigating differential drug susceptibility patterns for individual species within the *Mycobacterium avium* complex (MAC), which comprises *M. avium*, *M. intracellulare*, and closely related species such as *M. chimaera*, *M. colombiense*, *M. arosiense*, *among others.*
Objectives: To determine MIC distributions for *Mycobacterium chimaera*, *Mycobacterium intracellulare*, *Mycobacterium colombiense* and *Mycobacterium avium*, and to derive tentative epidemiological cutoff (ECOFF) values.
Methods: 683 non-duplicate bacterial isolates (*M. chimaera*, n = 203; *M. intracellulare*; n = 77; *M. colombiense*, n = 68; *M. avium*, n = 335) from 627 patients were subjected to antimicrobial susceptibility testing (AST) using the broth microdilution method. Tentative ECOFFs were determined by visual approximation and the ECOFFinder algorithm.
Results: For all tested compounds, MIC_{50} and ECOFF variations between the investigated species did not exceed one dilution step. Clarithromycin wild-type populations were mostly classified as susceptible (MIC_{50} = 2 mg / l, ECOFFs 8 to 16 mg / l; S ≤ 8 mg/l). Rifabutin MIC_{50} and ECOFF values were consistently lower than those of rifampicin (pooled dataset, 0.5 and 2 mg / l versus 8 and > 8 mg / l). Tentative breakpoints for moxifloxacin, linezolid and amikacin split wild-type populations of all species. ECOFFs for ethambutol, streptomycin, ciprofloxacin, isoniazid, trimethoprim/sulfamethoxazole and doxycycline were above the highest tested drug concentrations. Absolute agreement between the visually determined and the modelled 97.5 % and 99.0 % ECOFFs was 78.3 % and 65.0 %.
Conclusions: The *in vitro* drug susceptibility patterns of clinical *M. chimaera* isolates are comparable to those of other MAC isolates. Except for clarithromycin, current breakpoints for MAC categorization should be reevaluated. For collections of 50 to 300 isolates, statistical determination of the 99.0 % ECOFF may be superior to visual inspection of MIC distributions.
Population

P 14

The role of the IS6110 in micro- and macroevolution of *Mycobacterium tuberculosis* lineage 2

Egor Shitikov[1], A. Guliaev[1], J. Bespyatykh[1], Igor Mokrousov[3], E. Ilina[2], V. Govorun[1]
[1]*Federal Research and Clinical Centre of Physical-Chemical Medicine, Moscow, Russian Federation*
[3]*St. Petersburg Pasteur Institute, Russian Federation*

Members of the Mycobacterium tuberculosis complex contain the insertion sequence (IS) 6110 which, due to its high quantitative and positional variability, has become a widely used marker in epidemiological studies. The element plays an important role in microorganism genome plasticity, but still many consequences and causes of transposition have not been fully described. This work studies the transposition mechanism of IS*6110* and its impact on the evolution of *Mycobacterium tuberculosis* (*Mtb*).
Whole-genome sequencing data of 902 *Mtb* lineage 2 isolates was obtained from NCBI and ENA databases. Phylogenetic sublineages were determined based on SNP analysis (120 samples belonged to the ancient Beijing (17 proto-Beijing, 28 Asia Ancestral 1, 13 Asia Ancestral 2, 38 Asia Ancestral 3), 782 samples belonged to the modern Beijing (10 Asian African 1, 29 Asian African 3, 65 Asian African 2, 43 Pacific RD150, 140 Europe/Russia W148 outbreak, 361 Central Asia) (E. Shitikov et al., SciRep, 2017). ISMapper was used to determine the sites of integration of the IS*6110* (Hawkey et al., BMC Genomics, 2015).
We obtained 17972 points of insertion, which belonged to 865 independent positions in the H37Rv genome. The mean copy number per genome was 19.92 (from 9 to 25). To describe the evolution of an element in the genome, we arranged our samples in the order corresponding to a phylogenetic tree constructed on the basis of SNPs. We determined the stepwise mechanism of transposition, in which the transition to a new subpopulation is accompanied by a change in the localization of several copies of IS. It is important to note that the localization of the element in the ancestral population does not change, which implies a transposition only by «copy-paste» mechanism. In addition, we defined genes (537 sites (256 genes)) and intergenic regions (328 sites), where the element was integrated. Sixteen genes previously identified as being essential under different experimental conditions were found to contain IS. Further we carried out identification of IS*6110* mediated LSPs which showed the presence of recombination events (deletion) between inversely oriented elements.
In conclusion, we determined the evolution and role of IS*6110* for *Mtb* lineage 2 strains. We identified evolutionary and subpopulation-specific sites of integration which can be used for typing and subsequent research.
The work was supported by the grant of the Russian Fund for Basic Research RFBR 18-04-01035

P 40

Whole genome sequencing reveals transmission of tuberculosis between patient and guinea pig populations

Lesibana Malinga[1], Jeannette Brand[2], Elsabie de Kock[3], Peter van Heusden[4], Martie van der Wlt[1], Edward Nardell[5], Anton Stoltz[6]
[1]*South African Medical Research Council, Tb Platform; Department of Internal Medicine, University of Pretoria, South Africa*
[2]*South African Medical Research Council, South Africa*
[3]*University of Pretoria, South Africa*
[4]*University of Western Cape, Sanbi, South Africa*
[5]*Harvard Medical School, United States*
[6]*Department of Internal Medicine, University of Pretoria, South Africa*

Background: Transmission of *Mycobacterium tuberculosis* (*Mtb*) is a major problem in high burden countries. Traditionally transmission between case-pairs has been determined through molecular fingerprinting. Whole genome sequencing (WGS) with its potentially greater resolution could distinguish linked cases effectively and provide a greater insight genetic relatedness of strains allowing to study transmission in real-time. We therefore used a natural TB infection model using guinea pigs connected to a patient ward for investigating transmission under controlled conditions.
Materials and methods: Fifty-three patients (21 Females, 32 Males) with signs and symptoms of TB were admitted to an air facility in Witbank,

Mpumalanga for a period of 25 weeks. The patients were admitted for median hours (hrs) of 48 (IQR 12-552 hrs) while awaiting therapy and microbiological confirmation. A subset of nine patients were drug resistant. The air facility houses 360 guinea pigs continuously exposed to exhaust air of patient wards for a period of 4 months. Serial tuberculin skin tests (TSTs) were administered on guinea pigs to determine infection rates and sputum collected from patients on admission for microbiological (i.e microscopy and culture) and clinical investigations. Guinea pigs were euthanized after four months and their organs aseptically removed. Organs with lesions were processed for culture. DNA extracted from Mtb culture positives and WGS done at BGI (China). Phylogenetic analysis was done on PhyML 3.0. We used single nucleotide polymorphisms differences (SNPs) of 0-5 as evidence of transmission between human and animal case-pair.

Results: All patients had microbiologically confirmed Mtb infection using Xpert, microscopy and culture. Patients with available X-Ray readings had cavitary or lung infiltrates. We had 41 (11.4%) guinea pigs that were ≥ 6mm TST+ and of those six had histological evidence of TB lesions in lungs, liver, spleen and lymph nodes. These were all culture positive. We had WGS data for 21 (39.6%) and six (14.6%) patient and guinea pigs DNA respectively. We had three transmission case pairs between patients and guinea pigs. Transmission case pairs of patients with cavities and high smear grading (3+) had low SNP difference of 0-1.

Conclusion: Our study is likely to be the first to use WGS to document transmission between humans and guinea pigs. Due to limited patient isolates compared to animal TST positive rates we were unable to comment of clinical features associated with transmission. However, patients that transmitted happened to be cavitary, HIV negative and high smear grading.

P 43

Rapid and robust detection of the epidemiologically important Central Asian/ Russian strain of *M. tuberculosis* Beijing genotype

Igor Mokrousov[1], Anna Vyazovaya[1], Ekaterina Chernyaeva[2], Natalia Solovieva[2], Yuriy Skiba[3], Klavdia Levina[4], Violeta Valcheva[5], Weiwei Jiao[6], Lia Lima Gomes[7], Alena Gerasimova[1], Daria Starkova[1], Olga Narvskaya[2], Philip Noel Suffys[8], Marge Kütt[4], Adong Shen[6], Viatcheslav Zhuravlev[2]

[1]St. Petersburg Pasteur Institute, Russian Federation

[2]St. Petersburg Research Institute of Phthisiopulmonology, St. Petersburg State University, St. Petersburg, Russian Federation

[3]M.A. Aitkhozhin Institute of Molecular Biology and Biochemistry, Almaty, Kazakhstan

[4]North Estonian Medical Centre Foundation, Tallinn, Estonia

[5]Department of Infectious Microbiology, Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

[6]Beijing Key Laboratory of Pediatric Respiratory Infection Diseases, Beijing Pediatric Research Institute, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health; National Clinical Research Center for Respiratory Diseases; Key Laboratory of Major Diseases in Children, Ministry of Education, Beijing, China

[7]Oswaldo Cruz Institute, Fiocruz, Rio de Janeiro, Brazil

[8]Laboratory of Molecular Biology Applied to Mycobacteria, Oswaldo Cruz Institute, Fiocruz, Rio de Janeiro, Brazil; Insitute of Tropical Medicine in Antwerp, Belgium

Background*: Mycobacterium tuberculosis* isolates of the Beijing 94-32-cluster ('Central Asian/Russian strain') constitute an important component of the population structure of the pathogen in the Former Soviet Union countries; they are frequently associated with MDR/XDR and globally spread with immigrants. We sought to find reliable SNP marker(s) for this genotype and develop a simple method for its detection.

Methods: DNA of 19 Russian *M. tuberculosis* isolates of the Beijing genotype was subjected to WGS on the MiSeq platform (Illumina). The fastq and vcf files were submitted to comprehensive bioinformatics analysis. *M. tuberculosis* DNA collections available in the participating laboratories and previously characterized by 24-MIRU-VNTR and/or spoligotyping were used for the validation study.

Results: In total, 24 SNPs were found specific in the branch of isolates of the Beijing 94-32-cluster. We have chosen a mutation in *sigE* gene codon 98 CTG>CTA as candidate SNP for subsequent analysis and real-time PCR assay development. The specificity of this mutation was assessed through search in the GMTV database. The real-time PCR assay for allelic discrimination between wild type and mutants alleles was developed and optimized with DNA samples of the strains with available NGS data. Experimental validation of the real-time PCR assay was performed on the *M. tuberculosis* DNA collections representing Beijing genotype and non-Beijing families (342 isolates in total).

Conclusions: Based on whole genome

sequencing and in silico analysis, we identified a mutation *sigE* 98CTG>CTA specific of the Beijing 94-32-cluster. The developed real-time PCR assay may be useful for rapid detection of this epidemiologically relevant clonal group in prospective studies and for retrospective assessment of DNA collections in the Former Soviet Union countries and respective immigrant receiving countries.
Funding: Russian Science Foundation grant 14-14-00292.

P 52

Population structure of *Mycobacterium tuberculosis* in the Komi Republic, Russian Federation

Anna Vyazovaya[1], Eugenia Proshina[2], Snezhana Toinova[2], Ion Avadenii[3], Alena Gerasimova[1], Natalia Solovieva[3], Olga Narvskaya[3], Viacheslav Zhuravlev[3], Igor Mokrousov[1]
[1]St. Petersburg Pasteur Institute, St. Petersburg, Russian Federation
[2]Republican Anti-Tuberculosis Dispensary, Syktyvkar, Komi Republic, Russian Federation
[3]Research Institute of Phthisiopulmonology, St. Petersburg, Russian Federation

Introduction: The Komi Republic is located in the Norwest of the Russian Federation. It has a population of 900,000 and occupies 417 sq. km (density 2.02/sq.km). Most of it belongs to the Far North geographical area. A decrease in tuberculosis incidence from 56.4 to 35.3 / 100,000 in 2012-2017 was accompanied with sharp increase in XDR-TB rate among newly diagnosed patients from 2.3% до 15.9%. We aimed to assess the population structure of *M. tuberculosis* in this Russian region marked with low population density and harsh climate conditions.
Material and methods: A total of 90 *M. tuberculosis* isolates recovered in 2017 from newly diagnosed pulmonary TB patients were studied. *M. tuberculosis* culture and DST were performed using method of absolute concentrations and BACTEC MGIT 960. RIF and INH resistance gene mutations were tested using Amplitub real-time PCR system (Syntol, Moscow). The isolates were assigned to the Beijing genotype, Beijing B0-cluster and Beijing 94-32-cluster based on analysis of the specific markers: *dnaA-dna*::IS*6110*, *Rv2664-Rv2665*::IS*6110* and *sigE98* SNP, respectively. Non-Beijing isolates were subjected to spoligotyping followed by comparison to the SITVIT WEB.
Results: Half of the studied strains were drug resistant (44/90) whereas MDR were identified in 25 cases (of them 6 were XDR). Beijing genotype was detected in 48 isolates of which 14 (15.5% of all strains) and 31 (34.4%) were assigned to the B0/W148 и 94-32-clusters, respectively. These two major Beijing clusters differed significantly in the MDR rate: 71.4% and 29.0% (p = 0.02, OR = 6,11 [1,51; 24,66]). 42 strains of the non-Beijing genotype (3 were MDR) were represented by the T (n=13), LAM (n=12), Ural (n=9) and Haarlem (n=4) families; family status was 'unknown' in 4 isolates. The largest non-Beijing spoligotypes were SIT53/T (n=8), SIT52/T (n=4) and an Ural type (n=4) not present in SITVIT_WEB.
Conclusions: M. tuberculosis population in the Komi Republic, northwestern Russia is dominated by the Beijing genotype (53.3%) while three major non-Beijing families are found at the similar prevalence rates: T (14.4%), LAM (13.3%) and Ural (10.0%). MDR strains were more frequently found in Beijing B0/W148-cluster compared to the Beijing 94-32-cluster and MDR strains of these two clusters harbored rpoB Ser531Leu and katG Ser315Thr mutations.
Funding: Russian Science Foundation (project 14-14-00292).

P 54

Ancient sublineages of *Mycobacterium tuberculosis* Beijing family in West Siberia, Russia

Anna Vyazovaya[1], Oksana Pasechnik[2], Alena Gerasimova[1], Vladimir Stasenko[2], Igor Mokrousov[1]
[1]St. Petersburg Pasteur Institute, St. Petersburg, Russian Federation
[2]Omsk State Medical University, Omsk, Russian Federation

Background: The global population of the Mycobacterium tuberculosis Beijing family is known to be mainly represented by the strains of its modern sublineage. In contrast, strains of the ancient Beijing sublineages are more frequently found in East Asia, especially in southern China, Japan and Korea. In Russia, information on the ancient Beijing sublineages is scarce and their low prevalence at 5% was demonstrated only in northwestern European area of Russia. Here, we aimed to assess prevalence of the ancient Beijing strains in Omsk, Western Siberia and to compare the situation to the neighboring Russian and Kazakhstan provinces, in the light of historical and recent human migration.
Methods: A total of 423 M. tuberculosis strains were isolated from epidemiologically unlinked TB patients from Omsk region, West Siberia, Russia in 2011-2017. Beijing genotype was detected by

testing specific dnaA-dnaN::IS6110 insertion. Ancient and modern Beijing sublineages were detected based on analysis of NTF locus (intact in ancient strains). Analysis of RD181 deletion discriminated between “early ancient” and “classical ancient” Beijing strains with intact and deleted RD181, respectively. All ancient Beijing strains were subjected to spoligotyping and 24 loci MIRU-VNTR typing. The profiles were compared to the SITVIT_WEB and MIRU-VNTRplus databases.
Results: The Beijing family was identified in 280/423 (66.2%) isolates. Strains of the modern Beijing sublineage were detected in 85.7% (240/280). Strains of early and classical ancient Beijing sublineages were found in 3.9% (11/280) and 10.4% (29/280), respectively. Both ancient sublineages were dominated by MDR strains: 96.6% (28/29) and 100% (11/11), respectively. Interestingly, all early ancient Beijing strains belonged to spoligotype SIT269 and all classical ancient strains were SIT1. 24-MIRU-VNTR typing of all ancient Beijing strains revealed 11 variants (HGI 0.65), including 3 clusters. Early ancient subgroup was dominated by Mlva type 14717-15 (7/11; 63.6%). Classical ancient subgroup was dominated by Mlva type 1071-32 (23/29; 79.3%). The two ancient subgroups differed in 7 loci: MIRU10, 31, 39; QUB26, 4156; Mtub04, 39.
Conclusions: M. tuberculosis population of the Beijing family in the Omsk region in West Siberia is dominated by strains of the modern Beijing sublineage, but the prevalence of the ancient Beijing strains (15.3%) is triple than in northwestern Russia. Both modern and ancient Beijing strains in Omsk were characterized by high level of drug resistance, including MDR.
Acknowledgement. Russian Foundation for Basic Research grant #17-04-00367.

P 61

MIRU-VNTR typing of multi-drug resistant *Mycobacterium tuberculosis* complex clinical strains

Antonella Grottola[1], Giulia Fregni Serpini[1], Claudio Rabacchi[2], Maria Luisa Simone[2], Filippo Ferrari[1], Paola Pietrosemoli[1], William Gennari[1], Sara Tagliazucchi[1], Giulia Forbicini[1], Nadia Nanni[1], Rita Magnani[1], Monica Pecorari[1]
[1]Microbiology and Virology Unit, Modena University Hospital, Italy
[2]Department of Life Science, University of Modena, Modena, Italy

The species belonging to the *Mycobacterium tuberculosis* complex (MTC) are characterized by high genomic similarity. The MTC species can be defined by analysis of the regions of difference (RDs). The identified species can be classified into three principal genetic groups, PGG1b, PGG2 and PGG3, by single nucleotide polymorphisms (SNPs) analysis and can be distinguished in "Ancestral" and "Modern" through the presence/absence of the TbD1 region.
Even if *M. tuberculosis* is the main etiological agent of tuberculosis in humans, comparative analysis of entire genomes has allowed to define within the MTC seven human-adapted phylogenetic lineages and related sub-lineages characterized by different virulence, transmissibility and evolution in disease. These seven lineages comprise the obligate human pathogens *M. tuberculosis* Lineages 1-4, Lineage 7, as well as *M. africanum* Lineages 5 and 6.
The aim of this study is to complete the molecular epidemiological picture of the MTC multi-drug resistant (MDR) clinical strains, isolated between January 2009 and December 2017 in the Microbiology and Virology Unit of the Modena Polyclinic in Northern Italy by using MIRU-VNTR analysis. At this purpose 15 strains, previously characterized as Modern *M. tuberculosis* and belonging to the PGG1b (6/15; 60%), PGG2, (5/15; 33%), and PGG3 (4/15; 27%), were analyzed.
The bacterial DNA was extracted by boiling and ethanolic precipitation. The standard 24-locus based MIRU-VNTR typing was performed by using the available commercial kit following the manufacturer instructions (Genoscreen, Lille, France).
The MIRU-VNTR analysis showed a different profile for each strain. Five out of 15 strains (33.3%) had an undefined sub-lineage (n=4 PGG3; n=1 PGG2), 5/15 strains (33.3%) belonged to Beijing sub-lineage (n=5 PGG1b) and 5/15 strains belonged each to the single sub-lineage (6.6%), namely Ghana, URAL, TUR, Haarlem (n=4 PGG2) and Delhi/CAS (n=1 PGG1b) sub-lineages. Furthermore, the Neighbor Joining tree-based analysis showed that all the strains with undefined sub-lineage belonged to the Lineage 4/Euro-American. Considering that also the sub-lineages Ghana, URAL, TUR, Haarlem belong to the Lineage 4/Euro-American, this one results the most prevalent followed by the Lineage 2/East-Asian with the sub-lineages Beijing and the Lineage 3/East-African-Indian with the sub-lineage Delhi/CAS.
In conclusion, the study on the genomic diversity of the MTC-MDR strains showed considerable heterogeneity at level of lineages and sub-lineages. Moreover, these data show the prevalence in our geographic area of the Lineage 4/Euro-American and a remarkable presence of Lineage 2/East-Asian, both known to be widespread at global level and to have greater pathogenic power and transmissibility than other more geographically confined lineages.

P 88

Redefining an outbreak: Whole genome sequencing sheds light on the transmission dynamics of a multi-drug resistant *Mycobacterium tuberculosis* outbreak over 23 years

Anzaan Dippenaar[1], Rob Warren[1], Margaretha de Vos[2], Tim Heupink[3], Annelies van Rie[3], Charlene Clarke[1], James Posey[4], Samantha Sampson[1], Elizabeth Streicher[2]
[1]South African Medical Research Council Centre for Tuberculosis Research, DST/ NRF Centre of Excellence for Biomedical Tuberculosis Research, Stellenbosch University, Cape Towm, South Africa
[2]DST/NRF Centre of Excellence for Biomedical Tuberculosis Research, SA MRC Centre for Tuberculosis Research; Division of Molecular Biology and Human Genetics, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa
[3]University of Antwerp, Belgium
[4]Centres for Disease Control, United States

Background: Whole genome sequencing (WGS) has shown that *Mycobacterium tuberculosis* (*Mtb*) strains are more genetically diverse than previously assumed and that traditional genotyping methods cannot discriminate strain heterogeneity with high resolution which may mask their ability to accurately define the directionality of an outbreak.
Aim: To examine the evolution of a single *Mtb* cluster defined by a particular IS*6110* RFLP pattern to understand transmission and strain diversity over time.
Methods: Clinical *Mtb* isolates (n=97) with identical IS*6110* RFLP fingerprint patterns were selected from a longitudinal sample bank of *Mtb* isolates collected from a high tuberculosis incidence suburb in the Western Cape, South Africa from 1993 - 2016. DNA was extracted from *Mtb* cultures for WGS and analysis. Available WGS of *Mtb* isolates from surrounding suburbs were screened and 72 additional isolates from the same time period with the same genotype were included in the phylogenomic analysis. The genomic variants identified by WGS were used for phylogenomic inference, drug resistance prediction and to determine genomic distances between isolates.
Results: WGS analysis revealed unexpected genomic diversity within the seemingly homogenous IS*6110* cluster of *Mtb* isolates. Despite the IS*6110* RFLP based uniformity, at least six non-time dependent sub-clusters and several orphan-isolates were evident from the WGS-based phylogeny and genomic comparisons. Sub-clusters gained drug resistance conferring mutations (beyond MDR) on multiple occasions and *Mtb* isolates from surrounding suburbs were observed throughout the phylogeny.
Conclusion: IS*6110* RFLP typing underestimated the complexity of this 23 year outbreak. This study suggests that there is continuous circulation and reintroduction of this *Mtb* cluster in the community setting. Even with the advent of the WGS-era, confirming direct epidemiological links or outbreak directionality remains a challenge in high burden low income settings.

P 92

Purifying selective pressure suggests functionality of vitamin B12 biosynthesis pathway in a global population of *Mycobacterium tuberculosis*

Alina Minias[1], Piotr Minias[2], Bożena Czubat[3], Jarosław Dziadek[1]
[1]Institute of Medical Biology, Polish Academy of Sciences, Łódź, Poland
[2]Faculty of Biology and Environmental Protection, University of Łódź, Łódź, Poland
[3]Faculty of Biology and Agriculture, University of Rzeszów, Rzeszów, Poland

The functionality of vitamin B12 (cobalamin) synthesis pathway in *Mycobacterium tuberculosis* is under dispute and the ability to scavenge it from the host is unknown. Here, we quantified the ratio of non-synonymous and synonymous nucleotide substitution rates (dN/dS) in the genes involved in vitamin B12 biosynthesis, transport and cobalamin dependent enzymes in 3798 clinical strains of *M. tuberculosis*. Genes were tested for the presence of codon-specific signatures of negative or positive selection using DataMonkey online server for HyPhy package. Significant departure of codon-specific nucleotide substitution rates was tested with three different methods: Single Likelihood Ancestor Counting (SLAC), Fixed Effects Likelihood (FEL) and Fast Unconstrained Bayesian AppRoximation (FUBAR). We used $p < 0.08$ significance level to infer selection in FEL and SLAC analyses, while in FUBAR the cutoff limit to infer selection was set to 0.9 posterior probability. Codon-specific estimates of dN/dS ratios for each gene were obtained with MEGA software. The analysis on the level of individual codons, genes and groups of genes showed that the genes associated with vitamin B12 are predominantly under purifying selection that aims to maintain the most adaptive gene variants in the population. Further, these results suggest that biosynthesis pathway of vitamin B12 in *M. tuberculosis* is likely to be functional in most strains. However, since we

were able to find non-functional variants of several genes included the study, cobalamin synthesis does not seem to be essential for pathogen survival in clinical settings.

P 96

Whole genome sequence based resistance prediction and molecular typing of *Mycobacterium tuberculosis* complex (MTBC) strains in BioNumerics

Margo Diricks[1], Katrien De Bruyne[1], Hannes Pouseele[1]
[1]Applied Maths (Biomérieux), Sint-Martens-Latem, Belgium

Introduction: Tuberculosis (TB) is one of world's deadliest infectious diseases caused by the bacillus *Mycobacterium tuberculosis*. Whole-genome sequencing (WGS) has become an increasingly important tool for epidemiologic studies of TB, including outbreak detection and elucidation of infection sources and transmission routes. Furthermore, in contrast to the time-consuming phenotypic drug susceptibility tests (DST), WGS enables rapid prediction of associated antibiotic resistance, which is crucial for TB control and treatment. In this study, we present a new MTBC genotyping plugin for the BioNumerics software. This plugin can predict associated resistance to first and second line antibiotics, but also determines the species (within the genus *Mycobacterium*), the lineage and spoligotype of the strains under consideration.
Materials and methods: All functionalities within the MTBC plugin are based on sequence reads generated by WGS technologies. **Species decomposition** is based on the sequence of the 16S rRNA gene. The **lineage determination** component classifies the MTBC strains in 8 known lineages and 55 sublineages based on 62 single nucleotide polymorphisms. In addition, *in silico* **spoligotyping** is performed using the nucleotide sequences of 43 spacers as references in a local mapping analysis. Lastly, **resistance prediction** for 12 antibiotics is based on known mutations in 28 resistance genes. Additional resistance markers can be implemented in the plugin on a regular basis. The pipeline is integrated in the BioNumerics client software, and implemented on a scalable high-throughput calculation environment, providing results within 15 minutes upon submission. Results were validated on 161 MTBC samples (PRJNA187550).
Results: The majority (98%) of the strains were correctly identified by the plugin as being MTBC species and the predicted lineage of all samples corresponded with previous reports. For 99% of the MTBC isolates, the tool predicted the same spoligotype as the SpoTyping program. The sensitivity for isoniazid (INH), rifampicin (RMP), ethambutol (EMB), kanamycin (KAN) ranged between 80 and 95% while for ethionamide (ETH) this was only 50%. The specificity for INH, RMP, ETH, EMB and KAN, on the other hand, ranged between 70 and 100%. All samples were predicted to be resistant to streptomycin (SM) while this was only true in 52% of the cases according to phenotypic DST. The high amount of false positive and negative drug resistance predictions indicated the need for a revision or extension of the current genotype-phenotype correlations, which is currently being implemented.
Conclusion: The BioNumerics genotyping functionality for MTBC strains is an effective and user-friendly tool for species identification, strain typing and prediction of resistance and can thus be useful for high-throughput molecular surveillance and control of TB.

P 109

Phylogenetic assignment and drug resistance analysis of *Mycobacterium tuberculosis* from Mozambique and Brazil with Whole Genome Sequencing

Valdes Bollela[1], Cinara S. Feliciano[1], Livia M.P. Anselmo[1], Evangelina I. Namburete[1], Emilyn C. Conceição[2], Anzaan Dipenaar[3], Robin M. Warren[3], Christophe Sola[4], Wilson Araujo Jr.[1]
[1]School of Medicine of Ribeirão Preto, University of São Paulo, Brazil
[2]Instituto de Microbiologia, Universidade Federal Do Rio de Janeiro, Brazil
[3]DST/NRF Centre of Excellence for Biomedical Tuberculosis Research, Faculty of Medicine and Health Sciences, Stellenbosch University, South Africa
[4]Institut de Biologie Intégrative de la Cellule, Univ. Paris Sud, Université Paris-Saclay, France

Mozambique (MZ) and Brazil (BR) are included in the tuberculosis (TB) high-burden countries list from world health Organization, which accounts 85-89% of TB cases around the globe. Both nations belong to the Community of Countries with Portuguese speaking language and they had been related for more than four centuries mainly due to slave trade and immigration events from MZ to Southern BR. This study aimed to perform the phylogenetic assignment and drug-resistance (DR) analysis of *Mycobacterium tuberculosis* isolated from patients with pulmonary TB in Central-MZ and Southern-BR. A total of 134 isolates (73 from MZ and 61 from BR) were

sequenced by whole genome sequencing (WGS) after DNA extraction with CTAB. The WGS process included libraries preparation from pure DNA using Nextera XT DNA Library Preparation Kit and the sequencing was performed using an Illumina NextSeq instrument. For data analysis, we used BWA for mapping and SNIPPY for SNP calling. The final alignment was used to construct a Maximum Likelihood phylogeny using jModelTest. For DR mutations detection it was used TB-Profiler. In the Mozambican group four lineages were regnized: L1(38); L2(5); L3(1); L4(28); L6(1). Among the Brazilian isolates there were: L1 (1); L2 (5); L4(55). Based on WGS there were 27%(20/73) of multidrug-resistant TB in MZ in a two year period, while among the Brazilian isolates it was 32%(20/60) in a four year period. The most frequent mutations associated to rifampicin, izoniazid, pirazinamide, etambutol, streptomicin and floroquinolones were, respectively: rpoB- His445Tyr(MZ) and Ser450Leu(BR); KatG Ser315Thr (MZ/BR); pncA-Ala3Pro(BR) and Lys96Arg/Leu19Arg(MZ); emB-Met306Val and Met306Ile(BR), Gln497Arg(MZ); rrs intragenica(BR), rpsL Lys43Arg(MZ); gyrA Ala90Val(MZ/BR). This is the first study based on WGS to chacterize the genetic structure of *M. tuberculosis* isolates from MZ, by which compated to BR, it is more diverse. The presence of L6 in East of Africa is not common, but it was found in MZ.

Keywords: Phylogeny, Whole Genome Sequencing, Mozambique, Brazil.

P 128

Genetic diversity among *Micobacterium tuberculosis* in Mexico

Armando Martínez[1], Carlos Arturo Vázquez Chacón[1], David Vázquez -Gónzalez[1], Gabriela Espejel[1], Claudia Elena Bäcker[1]
[1]Laboratorio Supranacional de Tuberculosis, Instituto de Diagnóstico Y Referencia Epidemiológicos, Secretaría de Salud, Ciudad de México, México

Introduction: Tuberculosis (TB) still is an important public health problem in Mexico, for 2017 the incidence of pulmonary TB is 17.1 cases per 100,000 inhabitants. There is limited information about the genetic diversity of *M. tuberculosis* strains circulating in the country. In this work, 348 *M. tuberculosis* isolates collected in 29 different states of Mexico from 2015 to 2017 were retrospectively characterized by spoligotyping.

Materials and methods. 348 isolates collected in Mexico from 29 different states during the period 2008-2017 through the national TB surveillance as part of the national drug susceptibility Testing. All strains were isolated by the corresponding Local State Laboratory and subsequently submitted to the National Reference Laboratory by culture in Löwenstein-Jensen slants. Drug susceptibility testing was performed for first-line anti-tuberculosis drugs (streptomycin [S], isoniazid [H], rifampicin [R], ethambutol [E], and pyrazinamide [Z]) using MGIT-960 method and all *M. tuberculosis* strains were typed by spoligotyping.

Results: Drug susceptibility testing results were (14.4, 2.0, 1.4 and 0.5 % for H,R, E and Z, respectively). The subdivision of 205 MDR strains (59.7%) by combined drug resistance was as follows: only H and R, 50/348 (14.4%); combined resistance to three drugs, 26/348 (7.5%); resistance to four drugs, 94/348 (27%); and resistance to five drugs, 35/348 (10%). A total of 29 different spoligotypes were found. Among these, 59 (16.9%) were orphan strains obtained from 15 different states, were identified; 25.6 % strains corresponded to the T1 family represented the predominant pattern. The majority of the isolates belonged to the T and LAM families. Other minor families included the Beijing, EAI, S, U, X, H and *M. bovis* families. The discriminative power of the spoligotyping method, measured by the Hunter-Gaston index, was 0.9565.

Conclusions: The results showed that the TB lineages analyzed in this study exhibited a high degree of genetic diversity. The molecular characterization of *M. tuberculosis* isolates is likely to improve our understanding on the molecular basis in order to control TB transmission in the country. Our results highlight the importance of the implementation of a TB national molecular surveillance in Mexico.

P 152

Genome-based comparison of drug resistant *Mycobacterium tuberculosis* isolates causing pulmonary and extrapulmonary tuberculosis in Russia

Ekaterina Chernyaeva[1], Mikhail Rotkevich[2], Ksenia Krasheninnikova[2], Dmitriy Polev[2], Natalia Solovieva[1], Viacheslav Zhuravlev[1], Piotr Yablonsky[1], Stephen J. O'Brien[3]
[1]St. Petersburg Research Institute of Phthisiopulmonology, St. Petersburg State University, St. Petersburg, Russian Federation
[2]St. Petersburg State University, St. Petersburg, Russian Federation
[3]St. Petersburg State University, St. Petersburg, Russian Federation; Nova Southeastern University, Ft Lauderdale, Florida, United States

Background: The aim of the study is to conduct comparative analysis of whole-genome sequencing (WGS) data for clinical *Mycobacterium tuberculosis* strains collected from patients with pulmonary and extrapulmonary tuberculosis (PTB or XPTB, respectively) tissue localization.
Materials/methods: A total of 72 pulmonary and 73 extrapulmonary *M. tuberculosis* isolates were collected from different patients within the period from 2007 to 2014 in 40 different regions of the Russian Federation. Bacterial DNA was extracted and used for whole-genome sequencing (WGS) using MiSeq platform (Illumina). Sequencing reads were aligned to *M. tuberculosis* H37Rv reference genome (NC_000962.3) by using the bowtie2 program and for further single nucleotide variations (SNVs) calling and variant call format (VCF) file processing by using a combination of SAMtools, bcftools and VCFtools. VCF files were used for comprehensive genetic analysis.
Results: A comparative analysis of strains of *M. tuberculosis* revealed a high frequency of XPTB strains with extensive drug resistance. WGS data showed increased occurrence of Beijing genetic group among XPTB (82.2%) vs. PTB (66.7%) *M. tuberculosis* isolates. Phylogenetic analysis revealed *M. tuberculosis* genetic clusters associated with TB localization: XPTB is associated with Beijing CAO, A and 4.8 groups, and PTB with group 4.3. Analysis of SNVs did not allow to find genome markers, associated neither with pulmonary nor with extrapulmonary TB. HIV infection was shown to be significantly associated with the development of XPTB in the Beijing B0/W148 group and among unclustered Beijing. Analysis of mutations, associated with susceptibility to first and second line TB drugs allowed to reveal that relatively high proportion of isoniazid, rifampicin, streptomycin and ofloxacin resistant isolates had standard SNVs that used to predict drug-resistance profile.
Conclusions: Our research allowed making a snapshot of genomic markers identified in XPTB and PTB *M. tuberculosis* strains in Russia. We assume that further comprehensive analysis of bacterial and human biological signatures might allow for better understanding consistent pattern of XPTB development.
This work was supported by Russian Foundation for Basic Research Grant 16-34-60163

P 159

Characterization of *Mycobacterium tuberculosis* transmission dynamics by WGS in the metropolitan area of Rome

Angela Cannas[1], Ornella Butera[1], Carolina Venditti[1], Monica Sane-Schepisi[1], Antonio Mazzarelli[1], Gina Gualano[1], Fabrizio Palmieri[1], Antonino Di Caro[1], Enrico Girardi[1]
[1]National Institute for Infectious Diseases Irccs L.Spallanzani, Rome, Italy

Introduction: Improved control strategies are needed in order to limit transmission of tuberculosis (TB), also in low incidence countries. Although Italy has low rates of TB, significantly higher incidence can be found in areas with intense immigration, as the metropolitan area of Rome.
It is well known that molecular epidemiology can contribute to the characterization of TB transmission dynamics and to improve surveillance. Recently, the introduction of next-generation whole-genome sequencing (WGS) has provided a high resolution tool to conduct epidemiological studies.
The aim of our work was to characterize by WGS the TB strains recently circulating in the metropolitan area of Rome, and to provide information that can be helpful for implementing effective control strategies.
Methods: All TB strains isolated from patients consecutively admitted at the National Institute for Infectious Diseases in Rome between January 2016 and March 2017 with a diagnosis of TB, were subjected to WGS. Sequences were uploaded on SeqSphere+ (Ridom© GmbH) in order to generate a cgMLST-based minimum spanning tree and to identify clusters, and on PhyResSE 1.0 for lineage attribution. A further analysis was conducted including additional strains that were collected not consecutively from 2011 to 2015.
Results: Two-hundred-nine (209) *M. tuberculosis* isolates were collected and analyzed during the study. Of these, 153 (73.2%) were from foreign born (FB) patients, mostly (51%) from Romania. All geographic areas are represented except for Oceania. Lineage distribution shows >80% of Euro-American lineage in both, Italian and FB, with a majority of the Haarlem sub-lineage. Within the FB population lineages 1, 2 and 3 are well represented as EAI, Beijing and Delhi/CAS, respectively.
Twenty-nine (29) clusters were identified, involving 81 patients (39%). The majority (17/29) of them belong to the Haarlem sub-lineage and include a mixed Italian and foreign born population, mostly from Romania, followed by a large Euro-American lineage group (7/29), unclassified. Clusters of Beijing, EAI and Delhi/CAS are also found, in more homogenous populations. Most of the clusters are represented by drug-susceptible strains. A larger analysis, that adds 51 strains collected during 5 years preceding this study, increases the number of clusters, and shows a continuous transmission of TB in the area.
Conclusions: This study, conducted in a TB referral hospital setting in Rome using WGS,

shows recent spread of *M. tuberculosis*, mostly involving the Haarlem sub-lineage. Transmission between Italian and FB population is found, although this is limited to a well integrated ethnic group. Our results show a profile that is similar to what is observed in other metropolitan areas in Western Europe, and reflects the population movement profiles.

P 163

Molecular-genetic characterization of strains of non-tuberculous mycobacteria recovered in Ukraine in 2016-2017

Aleksand Zhurilo[1], Anna Barbova[1], Nataliia Kampos-Rodrihes[2]
[1]SI National Institute of Phthisiology and Pulmonology Named after F.G. Yanovsky of National Academy of Science of Ukraine, Ukraine
[2]State Institution "Public Health Center of the Ministry of Health of Ukraine", Ukraine

In recent times, in Ukraine, as everywhere else in the world, there is an increase in the number of cases of diseases caused by non-tuberculous mycobacteria (NTM), pathogens of mycobacterioses and tuberculosis-like lung diseases.
In the course of a routine epidemiological surveillance study on drug resistance in 2016-2017, we examined the issue of NTM distribution in Ukraine among patients suspected of tuberculosis (positive smear test result).
In recent years, mycobacteria have acquired new biochemical, enzymatic properties, and also some mycobacteria developed the ability to grow in nutrient media with various chemicals. Therefore, the existing phenotypic tests cannot always give an answer about the identification of circulating mycobacteria that are isolated from the sick.
Thus, using LPI analysis (DNA strip technology, Hain Lifescience CmbH, Germany) and GenoType Mycobacterium CM reagents, we conducted molecular genetic studies to identify isolates of mycobacteria that were recovered in a liquid nutrient medium in the MGIT BACTEC 960 system. GeneXpert MTB / RIF and the BD MGIT TBc ID immunochromatographic identification tests showed a negative result.
The results of the studies revealed that out of 1658 suspected of tuberculosis patients, 46 recovered non-tuberculous mycobacterium (2.8%).
Most non-tuberculous mycobacteria (NTM) - 45.7% of strains (21 isolates) were attributed to M. avium, 15.2% of strains (7 isolates) were identified as M.intracellulare, M.gordonae accounted for 13.0% (6 isolates), 10.9% of strains of mycobacteria (5 isolates) were classified as M. kansassi, M. malmoense consisted of 10.9% (5 isolates) and 4.3% of strains (2 isolates) were categorized as M. fortuitum.

P 179

Drug susceptibility and molecular characterization of *Mycobacterium tuberculosis* from Eastern Sudan

Yassir A. Shuaib[1], Matthias Merker[2], Eltahir Awad Khalil[3], Ulrich Schaible[2], Lothar H. Wieler[4], Mohammed Ahmed Bakheit[5], Saad El-Tiab Mohamed-Noor[6], Mohamed Abdelsalam Abdalla[6], Elvira Richter[7], Katharina Kranzer[2], Stefan Niemann[2]
[1]Research Center Borstel, Germany; Institute of Microbiology and Epizootics, Freie Universität Berlin, Germany; College of Veterinary Medicine, Sudan University of Science and Technology, Sudan
[2]Division of Molecular and Experimental Mycobacteriology, Research Center Borstel, Germany
[3]Institute of Endemic Diseases, University of Khartoum, Sudan
[4]Robert Koch Institute, Berlin, Germany
[5]Faculty of Veterinary Medicine, University of Khartoum, Sudan
[6]College of Veterinary Medicine, Sudan University of Science and Technology, Sudan
[7]Labor Limbach, Tuberculosis, Heidelberg, Germany

This study was conducted to investigate drug susceptibility and genotypes of *Mycobacterium tuberculosis* in Eastern Sudan. A total number of 383 sputum samples were collected from Kassala, Port Sudan, and El-Gadarif teaching hospitals from June to October 2014 and from January to July 2016. These samples were decontaminated by the NALC-NaOH method and cultured into MGIT liquid medium (Bactec MGIT 960 system) and onto Löwenstein-Jensen and Stonebrink slants for mycobacterial growth. A qPCR was conducted to verify the sensitivity of TB diagnosis using light microscopy in the study area. In addition, line probe assay (GenoType CM and MTBC), ITS gene sequencing, spoligotyping, MIRU-VNTR typing and whole generation sequencing (WGS) were conducted for genotyping and molecular characterization of the grown isolates. Culture of the specimens revealed growth of organisms from 196 (51.2%) of all samples. The majority of the isolates (n=171) were *M. tuberculosis* while the rest were either *M. intracellulare* (n=14) or mixed cultures with

M. tuberculosis and *M. intracellulare* (n=11). Any drug resistance was detected in 22.6% (40/177) of all of the *M. tuberculosis* isolates. Mono drug resistance was noted in 10.2% (18/177), MDR was in 10.2% (18/177), and poly-drug resistance was in 2.2% (4/177). However, neither extensively drug resistant nor totally drug resistant isolates were found. The combined spoligotyping, MIRU-VNTR 24-loci and WGS analysis showed that nearly three-quarters of the isolates belong to lineage 3 (Delhi/CAS genotype). In conclusion, there was an evidence of MDR transmission and acquisition. Lineage 3 isolates are responsible for causing the majority of TB cases.

Keywords: DST, genotype, tuberculosis, human, Sudan

P 185

Genomic epidemiology of a major outbreak of tuberculosis related to time, space, and virulence

Dorte B. Folkvardsen[1], Anders Norman[1], Åse Bengaard Andersen[2], Erik Michael Rasmussen[1], Lars Jelsbak[3], Troels Lillebaek[1]
[1]International Reference Laboratory of Mycobacteriology, Statens Serum Institut, Denmark
[2]Department of Infectious Diseases, Rigshospitalet, Denmark
[3]Department of Systems Biology, Dept of Biotechnology and Biomedicine, Technical University of Denmark

Denmark is a low-incidence tuberculosis (TB) country, but in the past decades, it has experienced an ongoing outbreak, documented with the genotyping methods Restriction Fragment Length Polymorphisms (RFLP) and Mycobacterial Interspersed Repetitive Units –Variable Number of Tandem Repeats (MIRU-VNTR). The outbreak has spread throughout the Danish Kingdom, including to the high-incidence country Greenland. In this study, the outbreak has been investigated by applying whole genome sequencing (WGS) to all strains in the period 1992-2014 typed to be Cluster 2 or 1112-15 (C2) with one of the genotyping methods. In total, 951 strains from 891 patients were included.
The aim was to decipher the outbreak based on WGS and investigate the new insight gained from applying this method to a previously characterized outbreak.
In addition to environmental factors, antibiotic resistance, and human genetics, there is increasing evidence that *Mycobacterium tuberculosis* (Mtb) strain variation plays a role for the outcome of infection and disease. We explored the reasons for the success of the C2 genotype by analyzing strain specific polymorphisms identified through WGS of all the isolates. Of 234 non-synonymous (NS) monomorphic SNPs found in C2 in comparison with Mtb reference strain H37Rv, 23 were in genes previously reported to be involved in Mtb virulence. Of these 23 SNPs, three were specific for C2 including a NS mutation in a gene associated with hyper-virulence. We show that the genotype is readily transmitted to different ethnicities. Our data suggest that strain specific virulence factor variations are important for the success of the C2 genotype. These factors, likely in combination with poor TB control, seem to be the main drivers of the C2 success. Furthermore, we constructed a list of SNPs found in genes associated with virulence. This list can be used as a tool for investigation of other successful outbreaks.
All these findings, suggest implications for TB control in Denmark and it is plausible that C2 is more virulent than the average Mtb strain, as it has caused outbreaks wherever it has been introduced throughout the Danish Kingdom, a kingdom consisting of both low- and high incidence settings.

P 194

Using TBminer to dig into 24-MIRU-VNTR and/or spoligotyping data of *M. tuberculosis* and the Whole-Genome Sequence era, the example of Pseudo-Beijing

Memona Yasmin[1], Jérôme Azé[2], Rubina Tabassum Siddiqui[1], Sabira Tahseen[3], Shahid Ahmad Abbasi[4], Naeem Khan[4], Rizwan Iqbal[5], Christophe Sola[6], Guislaine Refrégier[6]
[1]NIBGE, Faisalabad, PIEAS, Islamabad, Pakistan
[2]LIRMM, Université de Montpellier, CNRS, Montpellier, France
[3]National Tb Reference Laboratory, Islamabad, Pakistan
[4]Armed Forces Institute of Pathology, Rawalpindi, Pakistan
[5]Pakistan Medical Research Council, Lahore, Pakistan
[6]Institute for Integrative Biology of the Cell (I2bc), Cea-Cnrs-Université Paris-Sud, France

TBminer is an on-line classification tool using either spoligotype patterns, and MIRU-VNTR data. First version provided assignations of user strains according to most used taxonomies (SITVITWEB, MIRU-VNTR*Plus*, 1 to 7th lineages) and proposed a consensus classification. A second version is on the way to make explicit the correspondence with several SNP-based classifications.

We wanted to test whether TBminer was able to handle contradictory patterns. Pseudo-Beijing sublineage was discovered by Fenner *et al.* in 2011. It is characterized by a spoligotype profile identical to *bona fide* Beijing but associated to a smaller deletion in the CRISPR locus. MIRU-VNTR-based analysis indicates that it belongs to CAS lineage. We used a convenient sample from Pakistan, a country known to harbor a large diversity of CAS isolates. DNA was extracted by a CTAB procedure or by thermolysis. We performed 43-spacers Luminex spoligotyping and 24 MIRU-VNTR genotyping.
Out of the 225 Pakistanese isolates with complete genotypes, 8 isolates had a Beijing spoligotype profile (4%). Among them, 6 showed a 24 and a 15MIRU-VNTR patterns typical of Beijing lineage as analyzed independently both by TBminer (tool predicting MIRU-VNTR*plus* classification) and by MIRU-VNTR*plus* itself. In contrast, 2 other isolates were classified by both tools as CAS. These two isolates carried 2 copies of ETR-C repetition instead of 4 as in Beijing isolates, a feature present in Fenner *et al.* first Pseudo-Beijing isolates. TBminer was thus able to provide a reliable annotation for these isolates.
Altogether, we show that TBminer is an efficient tool to detect discordance between standard classifications' signatures. The ease of use of TBminer should help surveillance epidemiologists and clinical microbiologists to analyze more efficiently TB genetic diversity in their countries. This makes TBminer a useful tool to extract robust phylogenetic information from MIRU-VNTR and spoligotype patterns. In addition, we confirmed that in countries with high CAS prevalence, some isolates carrying a Beijing spoligotype may in fact be CAS isolates.

P 212

Assembling high-quality *Mycobacterium tuberculosis* complex genomes from short and long-read technologies

Miguel Blanco[1], Galo A. Goig[1], Manuela Torres[1], Irving Cancino[2], Iñaki Comas[1], Sebastien Gagneux[3]
[1]Biomedicine Institute of Valencia (CSIC-IBV), Tuberculosis Genomics Unit, Valencia, Spain
[2]Biomedicine Institute of Valencia (CSIC-IBV); Genomics and Health Unit, Fisabio Public Heath, Valencia, Spain
[3]Department of Medical Parasitology and Infection Biology, Swiss Tropical and Public Health Institute, Basel, Switzerland

Mycobacterium tuberculosis genome analyses are mainly based on mapping short-read sequences to a reference genome, typically H37Rv. However, mapping short-reads does not allow: i. to identify structural variations; ii. genetic variants in regions not present in the reference and iii. Identify variation in regions not accessible by short-read alignments. To identify the best assembly strategies for MTBC genomes we have analyzed several dataset generated by different technologies including Illumina (2x300bp), Nanopore MinION and PacBio data. First, we show that automatic assembly pipelines often generate inaccurate single-contig genomes. Second, we show that the best approach to generate high quality, closed genomes is a combination of short-read and long-read technologies together with recursive steps of quality control and refinement. We propose a pipeline based in de Bruijn graph assembly theory and manual curation of the draft genome. We apply this approach to the reconstruction of high-quality genomes from MTBC lineages with special interest on regions not accessible by short-read mapping and regions not present in the reference genome.

P 216

Nine-year molecular epidemiological study of tuberculosis in Hannover, Germany

Patrick Beckert[1], Mustafa Yilmaz[2], Ange Hanke-Lensing[2], Mareike Bliemeister[2], Silke Gerdes[3], Stefan Niemann[1]
[1]Molecular and Experimental Mycobacteriology, Research Center Borstel; German Center for Infection Research, Partner Site Hamburg-Lübeck-Borstel-Riems, Germany
[2]Region Hannover, Fachbereich Gesundheit, Hannover, Germany
[3]Region Hannover, Fachbereich Gesundheit, Hannover; German Center for Infection Research, Partner Site Hamburg-Lübeck-Borstel-Riems, Germany

Revealing transmission of *Mycobacterium tuberculosis* complex (MTBC) strains is crucial to guide efficient tuberculosis (TB) control strategies. To support the local health authorities in the contact tracing processes and get an in-depth understanding of TB transmission dynamics, we conducted a longitudinal molecular epidemiological study in Hannover, Germany.
MTBC strains of patients living in the Hannover region were collected from 2009 to 2017. After DNA extraction, molecular genotyping (24-loci MIRU-VNTR, spacer oligonucleotide typing, and whole genome sequencing) was performed. Phylogenetic lineage classification and clustering

was reported to the local health authorities.
406 patients were diagnosed with TB and included in the study. DNA of 286/406 MTBC strains (70 %) was further analyzed and comprehensive molecular genotyping data of 278/406 strains (68 %) could be obtained. The strains were classified into five MTBC lineages and *M. bovis*. 207/278 strains were assigned to lineage 4 (74 %), 32/278 strains to lineage 3 (12 %), 22/278 strains to lineage 2 (8 %), 8/278 strains to lineage 1 (3 %) and 3/278 strains to *M. africanum* lineage 6 (1 %). 6/278 patients (2 %) were infected with *M. bovis* strains. The strains of lineage 4 could be further classified into six sub lineages. Drug susceptibility testing revealed two patients being infected with MDR-TB strains, one classified as lineage 3 and one lineage 4.
The cluster analysis revealed that 77/278 patients (28 %) are grouped into 29 clusters ranging in size from two to eight patients. The largest cluster was caused by a lineage 4 strain; 4/8 patients were identified in 2012, 1/8 in 2013, 2/8 in 2014 and 1/8 in 2016. Additionally, two clusters each comprising five patients were identified. The strains of those clusters belonged to lineage 3 and 4. In the remaining 26 clusters, less than 3 patients were grouped. No transmission of MDR-TB strains was observed.
TB in the Hannover region is caused by strains belonging to five of the seven known MTBC lineages as well as by *M. bovis* strains. The majority of patients are infected with MTBC strains of the Euro-American Superlineage, lineage 4. Strains of this lineage 4 were grouped into the largest cluster including eight patients over a timeframe of five years. In addition, one cluster of five patients infected with strains of the lineage 3, originating in India and Central Asia was identified. Currently, whole-genome sequencing is conducted to improve the resolution of molecular epidemiology and provide more detailed information to the health authorities.

P 212

Current status of bovine tuberculosis in Poland – 8 years after the recognition of the country free of the disease

Lukasz Radulski[1]
[1]***National Veterinary Research Institute in Pulawy, Poland***

Bovine tuberculosis is an infectious disease of cattle with a strictly defined rules for the control and eradication. For many years Poland has struggled with bovine tuberculosis. In 2009, based on European Commission Decision of 23 April 2009, Poland has been recognized as officially free of bovine tuberculosis. This recognition does not mean, of course, complete elimination of the disease in veterinary practice. According to data for 2009, which recognized Poland as a country free from bovine tuberculosis, the study was performed including 111 samples from cattle suspected of being recognized as a disease (reactive tuberculin test). The existence of the disease was found in 60 animals from 12 farms (12 outbreaks). In another 2010 years the epidemiological situation deteriorated slightly: a total of 147 samples were tested, and the disease was found in 18 different farms. Next year 2011 was similar: a total of 180 samples were tested and the existence of tuberculosis reported in 16 outbreaks. In 2012 it were 15 outbreaks, in 2013 – 22, 2014 – 16, 2015 – 31, 2016 – 33 and in 2017 - 12. Majority of outbreaks in year 2009-2015 were located in the northern part of the mazowieckie province, where for many years the existence of outbreaks of bovine tuberculosis was observed. In 2015-2017, the location of tuberculosis centers has changed. Now most of them are in the wielkopolskie province. Decision of the Chief Veterinary Officer shall be tested annually 20% of the cattle in the area so that within 5 years the entire population was examined. In laboratory test the negative rate (without isolation of strain) ranged from 40 to 50%. This indicates a significant proportion of false-positive results ascertained tuberculin test. Certain diagnostic facilities introduce the possibility of using the interferon gamma test, although it has similar limitations as the single intradermal tuberculin test. Taking into account all the data obtained during the period, it is clear that testing for bovine tuberculosis in Poland and the adopted system allows to maintain the achieved status of a bovine tuberculosis-free and allows for controlled combat and further elimination of the disease in cattle herds.

P 28

Transmission of bovine tuberculosis among wild animals in Southern Poland

Lukasz Radulski[1]
[1]***National Veterinary Research Institute in Pulawy, Poland***

Bovine tuberculosis (BTB) is one of the most import bacterial diseases among wild animals. Wild boar BTB has already described in Europe, in Bulgaria, Croatia, France, Germany, Hungary, Italy, Portugal, Slovakia, Spain and the United Kingdom as the main wildlife reservoir for tuberculosis. Currently, Poland from 2009 is recognized as officially free of the disease. However (BTB) cases are rarely found in domestic and wild animals. For example in November 2012, the dead wild boar (female, ca 4 years old) was found in the Bieszczady Mounatains. In the same area for many years the existence of bovine tuberculosis in bison (European bison) was observed. In the same year, tuberculosis infections in other wild boar were also found. The spoligotyping and MIRU-VNTR analysis proved that the infection caused the same M. bovis ssp. bovis strain. In the following years 2013-2017, the epidemiological situation in the field of bovine tuberculosis was monitored among these wild animal species. The dissected tissue material from wild boars and bisons dead or sanitary shooted in this area was soaked and homogenized in a 5% solution of oxalic acid. The resulting supernatant was removed and the pellet was washed twice with sterile physiological saline. The rinsed pellet for inoculation of culture media - 3 slants of Stonenbrink's, Petragnani's and Loewenstein – Jensen's media was used and incubated at 37°C. for 42 days. In total, in the years 2012-2018 (until 15/02/2018), samples from 118 bison and 50 feral pigs were tested. In total, 35 positive results were found among samples from bison, which is 29.6% and from wild boars 32 (64.0%). Especially the high intensity of infection was in the years 2012-2016. In the years 2017 - 2018, no bovine mycobacteria were found in any of the 28 tested samples from the bison. In the same period, only 1 wild boar was tested, also with negative results. The small

number of samples from wild boars results from restrictions caused by the eradication of African swine fever. In the end, it was not proved if wild boars were infected from bisons or vice versa. Thus, despite the elimination of the sick bison herd, the control of tuberculosis in this area can be very difficult.
Based on the obtained results, it can be concluded that the peak of infection took place in 2012-2016. In the last several months of observation, no positive results in the direction of BTB were found, which indicates that the infection is extinguished. Despite the good epidemiological situation, control of the spread of bovine tuberculosis between wild animals and in cattle herds is closely monitored

P 70

Evaluation of Interferon gamma test as one of the eradication program for bovine tuberculosis in Korea

Yun-Ho Jang[1], Tae-woon Kim[1], Min Kyu Jeong[1], Yu sin Ok[1], Bang-Hun Hyun[1], Jae Myung Kim[1]
[1]Bacterial Disease Division, Animal and Plant Quarantine Agency, Gimcheon, Gyeongsangbuk-do, Korea

Purpose: The objective of this study is to assess the IFN-r assay in terms of diagnostics and as large scale surveillance program in Korea
Materials and methods: From 2013 to 2016, we performed the IFN-γ test to cattle (n=120) having bovine tuberculosis (bTB) gross lesion and/or culture positive to evaluate the sensitivity and additionally performed to cattle from bTB free herds (n=426) to evaluate the specificity depending on various cut-offs. And since 2013, we collected the IFN-γ and CFT (Caudal fold test) data from 9 provinces and 8 metropolitan cities in South Korea to compare the results of two tests. And serial and parallel application of the IFN-r and skin test to bTB control program was compared by using samples (n=91) identified as bTB infected by laboratory tests.
Results: When cut-off (OD_{PPD-B}-$OD_{PPD\ A}$ and $OD_{PPD\ B}$-OD_{PBS}) optical density (O.D) was 0.1, sensitivity and specificity were 81.7% (98/120) and 99.5% (424/426). And when cut-off O.D was 0.05, it showed quite higher sensitivity (86.7%, 104/120), but lower specificity (98.4%, 419/426) than when OD 0.1. Overall agreement between the IFN-r and CFT (n=340,133) was 98.1%, and Cohen's kappa value of two methods was 0.478, which was indicating moderate agreement. Conditions on the IFN-r and/or CFT were applied to control program, bTB detection rates for serial, single (CFT), single (IFN-r) and parallel were 47.2% (43/91), 63.7% (58/91), 78.0% (71/91) and 94.5% (86/91).
Conclusions: The IFN-γ test to date is mainly used as an ancillary test in explosive TB breakdowns or persistently infected herds alongside the skin tests. Our study showed that the IFN-r and CFT are complementary each other, although both are targeted to cellular immune response. Therefore, parallel application of two tests is considered as most helpful approach to reduce bTB prevalence in high endemic regions.

P 72

Nested-PCR for detection of *Mycobacterium bovis* in herd environmental samples

Tae-woon Kim[1], Yun-Ho Jang[1], Min Kyu Jeong[1], Yun Jeong Seo[1], Bang-Hun Hyun[1], Jae Myung Kim[1]
[1]Bacterial Disease Division, Animal and Plant Quarantine Agency, Gimcheon, Gyeongsangbukdo, Korea

Background: The detection method *M. bovis* from the environmental samples is needed to control bovine tuberculosis (bTB) on herd level. In this study, we attempted to establish a method for detection of *M. bovis* in environmental sample using nested-PCR method and to detect residual *M. bovis* in bTB outbreak herds in South Korea.
Materials and Methods: 142 Environmental samples from 27 herds where have a history of bTB outbreak and 83 Environmental samples from bTB free herds (n=15) were used for the detection of residual *Mycobacterium bovis.* Amplicon libraries of *IS 6110* target gene fragment was amplified from *M. bovis* DNA by using the two types of primer sets. For the first step, PCR amplification of the 716 bp length for *IS 6110* gene was performed and each sample was purified to remove the others. The purified library amplicons were re-amplified by second PCR primer set (203 bp) to increase detection sensitivity.
Result: The results of detection of residual *M. bovis* on environmental sample, 72.5% of fecal samples (105/120) DNA were shown positive band by nested PCR method and 81% (18/22) samples of water samples were detected as positive. In the bTB free herds, only 7 out of 15 farms were shown negative results. 19% (16/83, fecal sample 14/68, water sample 2/15) samples showed positive results. The results of the detection of *M. bovis* using Nested PCR method were found to be higher than that of the commonly used PCR method.
Conclusion: The sensitivity and specificity of two methods based on the polymerase chain reaction (PCR) for detection of *M. bovis* were

compared. In the standard PCR method, the sensitivity of detection of *M. bovis* was very low due to contaminants and other microorganisms in environmental samples. However, the sensitivity of this nested-PCR for *M. bovis* was high compared to previous one, which is useful as a detection method in environmental samples. However, because bTB is endemic and cattle movement between herds and regions is frequently in South Korea. Therefore, more study about whether some positive results from bTB free herds indicate false positive or not is needed with culture results using environmental samples to apply this Nested PCR to screening method.

P 224

Serological response from early and long cultures of *Mycobacterium bovis* isolates from Zacatecas, Mexico: Potential biomarkers for TBb diagnostic

Javier Jovani Sanchez Garza[1], Jua Manuel Favela Hernandez[2], Isaias Balderas Renteria[3], Blanca Patricia Lazalde Ramos[4], Sol Maria Quirarte Baez[5], Gloria Guillermina Guerrero Manriquez[1]

[1]Lab de Bioquímica Molecular e Inmunobiologia. Unidad Académica de Ciencias Biológicas. Campus II. Uaz. Zacatecas, Zac, Mexico

[2]Instituto Multidisciplinario de Investigación. Avicena, A.C. Torreón, Coahuila, Mexico

[3]Lab de Ing. Genética Y Genómica. Departamento de Investigación Y Posgrado de la Fac de Química, Uanl. Monterrey Nvo León, Mexico

[4]Unidad Académica de Ciencias Químicas. Campus Siglo XXI. Uaz, Mexico

[5]Coordinacion de Planeación Y Enlace Institucional, Delegación Zacatecas, Imss. Zacatecas, Mexico

Bovine tuberculosis (bTB) is a chronic infection disease caused by *Mycobacterium bovis* (*M. bovis*) a member of the *M. tuberculosis complex*. bTB is a threaten for animal and human health. It has been described that more than 50 millions of cattles are infected worldwide with *M. bovis*, resulting in 3 billion losses annually worldwide. Ddespite of multiple efforts, there is a complete absence of alternatives for control and eradication programs against bTB. Prophylactic and therapeutic strategies are not common practice in cattles, because of the risk of multidrug resistant appereance strains In the last decades, molecular techniques based in multiplex PCR are a promising alternative toward a more precise and viable bTB diagnostic test. However, to develop a sensitive PCR based assay as a diagnostic routine tool, it is critical to consider the quality of the DNA, the primer and the amplification region of the mycobacteria. In the establishment and the development of the active disease, the complex of Mycobacteria depends of the release of the mycobacterial antigens, and this is constitute a key prerequisite for strong immune responses and determine the outcome of the infection, because their immunomodulatory role in the host. Pessolani et al., reported that there were a serological response of a doublet of proteins from early cultures of *Mycobacterium bovis* BCG, of low molecular weight, toward leprosy patients serum. There are other mycobcterial antigens that has a role as a virulence factors. But still remains to be defined their role in the immune pathogenesis of bTB. Therefore, in the present work, we pursued to investigate the antigens released to the culture medium of *M. bovis* isolates from Zacatecas, Mexico at early (7 days) and long period of time (21 days). *M. bovis* identity was confirmed by PCR amplification of 370 pb fragment corresponding to the RD1 region. The electrophoretic patterm rofile of early (seven) and long (21) days released antigens were recovered by centrifugation and analyzed in 12% SDS-PAGE stained with Blue Coomassie. Enrichment of proteins in the supernatant of these cultures was made by precipitation with ammonium sulfate. The resultant proteins were transferred to nitrocellulose membrane. Serological response was analyzed using a pool of negative and positive serum from Zacatecas and Coahuila, Mexico. It was found that proteins of around 60-80 kDa were mostly recognized by the serum of infected animals. These data could account as biomarkers to develop a more precise, specific bTB diagnostic.

AUTHOR INDEX