38th

Annual Congress
of the European Society
of Mycobacteriology

ESM

25th – 28th June 2017
Šibenik, Croatia

Scientific Program including Abstracts

Impressum

Publisher
Agency KONSENS Ltd.
Stockumer Straße 30
59368 Werne
Germany
Phone: +49 23 89 / 52 75 0
Homepage: www.agentur-konsens.de

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Design & Layout
Agency KONSENS Ltd.

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WELCOME MESSAGE

Dear Friends and Colleagues,

with great pleasure and honor, we announce that the 38th meeting of the European Society of Mycobacteriology will be held in Šibenik, Croatia from June 25th to 28th 2017.

Croatia lies on the eastern coast of the Adriatic Sea which is a part of the Mediterranean Sea. The narrow Dinara Mountain Range separates the country's Mediterranean region from its central European continental part, which spans from the edges of the Alps in the North West to the shores of the Danube in the East, encompassing the southern part of the Pannonian lowlands.

In the middle of the Croatian coast where the Krka River enters the sea, lies Sibenik, the oldest native Croatian city in the Adriatic. Nowadays the center of Šibenik-Knin County, Šibenik was first mentioned in 1066. During the 15th and 16th century, it was one of Croatia's most important renaissance centers with many architectural monuments originating from that period. The crystalline waters of the Šibenik region include 242 islands, islets and above-sea reefs of splendid beauty with the breathtaking, unspoiled landscape of the Kornati archipelago.

The disturbing data presented in the new 2016 WHO report show the increase in TB morbidity and mortality, emphasizing even more the need to exchange the knowledge and the experience in fighting TB. The scientific program of the 38th 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.

We hope that you will equally enjoy the scientific part of the meeting, as well as natural beauties and exquisite gastronomy – all of that in good company!

Looking forward to see you all in Šibenik, Croatia.

The Scientific Committee

Daniela Maria Cirillo
Vera Katalinić-Janković
Ljiljana Zmak
Stefan Niemann

CONGRESS ORGANIZATION

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SCIENTIFIC PROGRAM

Sunday, 25.06.2017

12:00 **Registration**

14:00-16:00 **ESM Workshop on WGS for diagnosis and surveillance**
Chairs: *Daniela Maria Cirillo, Stefan Niemann*

NGS from sputum
Philipp Supply (France)

UK NGS resistance prediction
Timothy Walker (UK)

NGS Based Solutions for M.tuberculosis Surveillance and Diagnosis – Foundations and Realization
David L. Dolinger (USA)

Update on free database
Stefan Niemann (Germany)

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

16:00-16:20 **Symposium 1: HAIN LIFESCIENCE GmbH**
Chair: *Stefan Niemann*

16:20-16:40 **Symposium 2: BECTON DICKINSON GmbH**
Innovating Tuberculosis Diagnostics: Then and Now - Early Clinical Results for the BD MAX MDR-TB Assay
Salman H. Siddiqi, PhD
Salma Kodsi, MBA, CCRA - Director, Medical Affairs & Clinical Evidence, BD Life Sciences

16:40-17:00 **Symposium 3: CEPHEID EUROPE SAS**
Chair: *Paolo Miotto*

Next generation tuberculosis diagnostics: Test and Treat
Elisa Tagliani (Italy)

17:00-17:30 **Coffee break and visit of the exhibition**

18:00-18:25 **Opening session:**
Congress Presidents:
Daniela Maria Cirillo, Vera Katalinić-Janković, Stefan Niemann

18:25-18:30 **Travel Grant Awards**
Chair: *Daniela Maria Cirillo*

18:30-19:15 **Gertrud Meissner Award ceremony**
Chair: *Daniela Maria Cirillo*
Emergence and spread of multidrug resistant tuberculosis in Eurasia ("Combat an old enemy with new weapons")
Lecture of Matthias Merker

19:15-19:45 ***Gardner Middlebrook Award ceremony***
Chair*: Stefan Niemann*
Lecture of the laureate

19:45 **Welcome reception at the *Legend Bar***

Monday, 26.06.2017

Morning

9:00-10:45 **Research needs to achieve eradication of tuberculosis**
Chairs: *Claudio Köser, Vera Katalinić-Janković*

9:00-09:45 Guest lecture 1: Global Priorities in tuberculosis research
Daniela Maria Cirillo (Italy)

9:45-10:00 pncA mutations in Mycobacterium tuberculosis is a strong predictor of poor treatment success in the therapy of multidrug resistant tuberculosis (OP 1)
Yi Hu

10:00-10:15 Evaluation of Deeplex-MycTB assay for the identification and antibiotic susceptibility profiling of Mycobacterium tuberculosis strains from primary specimens (OP 2)
Elisa Tagliani

10:15-10:30 Widespread use of inappropriate MTBDRplus PCR conditions impacts performance (OP 3)
Grant Theron

10:30-10:45 What is resistance? Impact of molecular drug resistance testing on multi- and extensively drug-resistant tuberculosis therapy (OP 4)
Matthias Merker

10:45-11:15 **Coffee break and visit of the exhibition**

11:15-13:00 **Research needs to achieve eradication of tuberculosis**
Chairs: *Troels Lillebæk, Daniela Maria Cirillo*

11:15-12:00 Guest lecture 2: Breakpoints for new and old anti tuberculosis drugs
Claudio Köser (United Kingdom)

12:00-12:15 A bespoke dry broth microdilution panel for susceptibility testing of M. tuberculosis: a validation study (OP 5)
Ana Luíza Gibertoni Cruz

12:15-12:30 ABashTheBug.net: A citizen science project helping to classify M.tuberculosis drug susceptible tests (OP 6)
Philip Fowler

12:30-12:45 The correlation of phenotypic and genotypic resistance of Mycobacterium tuberculosis on different levels of drug concentration (OP 7)
Valeriu Crudu

12:45-13:00 Evaluation of Abbott RealTime MTB INH/RIF Resistance Assay using well-characterized M. tuberculosis Isolates from Central Asia (OP 8)
Sabine Hofmann-Thiel

13:00-14:00 **Lunch and visit of the exhibition**

Afternoon

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

15:45-16:30 **Research needs to achieve eradication of tuberculosis**
Chairs: *Stefan Niemann, Alena Skrahina*

15:45-16:00 Guest lecture 3: Tuberculosis: the impact of lesion diversity on drug penetration and sterilization
Veronique Dartois (USA)

16:00-16:15 Personalized treatment for tuberculosis: lessons from a single case over eight years of disease (OP 10)
Iñaki Comas

16:15-16:30 Combination of antimicrobial peptides and isoniazid/rifampicin against multidrug-resistant tuberculosis (OP 11)
DeDe Man Kwun Wai

16:30-17:00 Coffee break and visit of the exhibition

17:00-18:15 Research needs to achieve eradication of tuberculosis
Chairs: *Stefan Niemann, Alena Skrahina*

17:00-17:45 Guest lecture 4 : New drugs in action: case reports
Alena Skrahina (Belarus)

17:45-18:00 Evaluation of whole genome sequencing for drug susceptibility testing of Mycobacterium tuberculosis (OP 12)
Janko van Beek

18:00-18:15 Use of Whole Genome Sequencing approach for diagnosis of drug-resistant tuberculosis in a routine setting (OP 13)
Andrea Maurizio Cabibbe

18:30 Departure Boat trip

20:00 Traditional dinner at the *Ethno Village* in Solaris Beach Resort

Tuesday, 27.06.2017

Morning

9:00-10:45 New concepts on LTBI and progression
Chairs: *Nalin Rastogi, David L. Dolinger*

9:00-9:45 Guest lecture 5: Protocols for validating new tools for latent tuberculosis
Alberto Matteelli (Italy)

9:45-10:00 Single-live imaging system for the analysis of in vitro/ex vivo macrophage infection models for tuberculosis (OP 15)
Paolo Miotto

10:00-10:15 Genetic and functional analysis of polymorphisms in the PKLR gene and association with leprosy and tuberculosis (OP 16)
Ohanna Bezerra

10:15-10:30 Contribution of efflux pumps to Rifampicin resistance in clinical isolates of M. tuberculosis (OP 17)
Mandira Varma-Basil

10:30-10:45 HIV and tuberculosis in an intermediate-incidence country - a retrospective study at a Portuguese Infectious Diseases Department (OP 18)
Joana Alves

10:45-11:15 Coffee break and visit of the exhibition

11:15-13:00 New concepts on LTBI and progression
Chairs: *Alberto Matteelli, Veronique Dartois*

11:15-12:00 Guest lecture 6: Towards signatures for tuberculosis progression
Hazel Dockrell (United Kingdom)

12:00-12:15 Differential No-PDIM Lipid Production in rifampicin-resistant and H37Rv Mycobacterium tuberculosis strains: search of rifampicin .resistant Biomarkers (OP 19)
Edgar Rodríguez-Beltrán

12:15-12:30 Association between interleukin-2, interleukin-4 gene polymorphisms and pulmonary multi-drugresistant tuberculosis (OP 20)
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12:30-12:45 Comprehensive genome-wide analysis of Mycobacterium tuberculosis strains isolated from patients with tuberculous spondylitis (OP 21)
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12:45-13:00 Unravelling the transcriptome of phenotypically different isogenic cells (OP 22)
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15:30-17:15 Tuberculosis in children and other relevant topics
Chairs: *Sebastian Gagneux, Dick van Soolingen*

15:30-16:15 Guest lecture 7: Tuberculosis in children what is new from diagnosis to therapy
Steven Welch (United Kingdom)

16:15-16:30 Population Structure and Clinical Presentation of Pediatric Mycobacterium tuberculosis in Florida, 2009-2015 (OP 23)
Marie Seraphin

16:30-16:45 Spatiotemporal analysis of animal-adapted members of Mycobacterium tuberculosis complex: epidemiology connections revealed by fine-scale genome analyses (OP 24)
Ana Reis

16:45-17:00 Time-and-Motion tool for assessment of working time in tuberculosis laboratories: a multi centre study (OP 25)
Vladyslav Nikolayevskyy

17:00-17:15 European TB Laboratory Initiative: Algorithm for laboratory diagnosis and treatment-monitoring of pulmonary tuberculosis and drug-resistant tuberculosis using rapid molecular diagnostic technologies (OP 26)
Soudeh Ehsani

17:15-18:00 General assembly

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Morning

9:00-11:00 Population structure and transmission of mycobacteria
Chairs: *Iñaki Comas, Sven E. Hoffner*

9:00-9:45 Guest lecture 8: Population structure and transmission of M.tuberculosis
Sebastian Gagneux (Switzerland)

9:45-10:00 Genomic epidemiology spanning decades of major Mycobacterium tuberculosis outbreak in a low-incidence setting: Lessons from sparse time-series analysis of a retrospective cohort study (OP 27)
Anders Norman

10:00-10:15 VNTR typing is less reliable in predicting epidemiological links than expected, as indicated by Whole Genome Sequencing (WGS) analysis (OP 28)
Rana Jajou

10:15-10:30 Findings from Whole Genome Sequencing of Tuberculosis in a Geographically Large Canadian Province with a Diverse Population (OP 29)
Jennifer Guthrie

10:30-10:45 WGS analysis of clusters involving immigrants to develop tailored molecular tools to discriminate between recent transmission in the host country and new importations (OP 30)
Dario Garcia de Viedma

10:45-11:00 Evolution of Extensively Drug-resistant Mycobacterium tuberculosis in Western Cape Province of South Africa: Whole Genome Sequencing of Serial Patient Isolates (OP 31)
Serej Ley

11:00-11:30 Coffee break and visit of the exhibition

11:30-13:30 Population structure and transmission of mycobacteria
Chairs: *Liliane Mack, Matthias Merker*

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Thomas A. Kohl (Germany)

12:15-12:30 The Phylogeny and Taxonomy of the Genus Mycobacterium (OP 32)
Conor Meehan

12:30-12:45 Nocosomial outbreak of NTM of the Mycobacterium fortuitum complex – Detection of transmission by Whole Genome Sequencing depends on a reduced set of core division proteins (OP 33)
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12:45-13:00 Mycobacteria in aquarium fish: poor awareness of their zoonotic potential (OP 34)
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13:00-13:15 Exploring the clinical relevance of NTMS causing pulmonary and extra pulmonary diseases in Saudi Arabia: Findings of the first national surveillance study.
Exploring the clinical relevance of NTM mycobacteria causing pulmonary and extra pulmonary diseases in Saudi Arabia (OP 35)
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13:45-14:00 Closing remarks

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L 2
UK NGS resistance prediction
Timothy Walker

L 3
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David L. Dolinger

L 4
Update on free database
Stefan Niemann

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GL 2
Breakpoints for new and old anti tuberculosis drugs
Claudio Köser

GL 3
Tuberculosis: the impact of lesion diversity on drug penetration and sterilization
Veronique Dartois

GL 4
New drugs in action: case reports
Alena Skrahina

GL 5
Protocols for validating new tools for latent tuberculosis
Alberto Matteelli

GL 6
Towards signatures for tuberculosis progression
Hazel Dockrell

GL 7
TB in children what is new from diagnosis to therapy
Steven Welch

GL 8
Population structure and transmission of M. tuberculosis
Sebastian Gagneux

GL 9
High-resolution analysis of M. abscessus and M. chimaera transmission dynamics and population structure by whole genome sequencing
Thomas A. Kohl

Gertrud Meissner Award

Emergence and spread of multidrug resistant tuberculosis in Eurasia
(“Combat an old enemy with new weapons”)
Matthias Merker

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A bespoke dry broth microdilution panel for susceptibility testing of M. tuberculosis: a validation study
Ana Luíza Gibertoni Cruz on behalf of Comprehensive Resistance Prediction for Tuberculosis: an International Consortium Members

OP 6
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Philip Fowler on behalf of Comprehensive Resistance Prediction for Tuberculosis: an International Consortium members

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New concepts on LTBI and progression

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Tb in children and other relevant topics

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Population structure and transmission of mycobacteria

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ABSTRACTS OF LECTURES (L)

L 1

NGS from sputum

Philipp Supply

L 2

UK NGS resistance prediction

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L 3

NGS Based Solutions for M.tuberculosis Surveillance and Diagnosis – Foundations and Realization

David L. Dolinger

In order to address issues there needs to be a detailed understanding of their range and magnitude. Do we truly understand the extent of the issues concerning the control of tuberculosis (TB) and the information gaps which need to should be filled? One key to the effective control of TB and the spread of drug-resistant (DR-TB) strains is accurate information pertaining to the drug resistance, susceptibility and general surveillance patterns. There are currently available platforms and platform based solutions which can be utilized. However, there has not been a focused effort to implement such solutions partially because funders do not understand the need and assume that industry or others will fill the need that they perceive, a lack of an assay. In addition, the focus has not been on the whole – an end-to-end solution but only on the pieces and parts. Next generation sequencing (NGS) has the potential to affectively change global health and management of TB. While industry has focused primarily on using NGS for oncology diagnostics and human genomics, the area where NGS can rapidly impact healthcare is in the area of infectious disease surveillance and diagnostics in low and middle income countries (LMICs). To date as a community, there has been a failure to capitalize on the potential of NGS, especially where it can provide actionable information pertaining to treatment options for patients at the reference laboratory level. The rapid evolution of knowledge about the genetic foundations of TB drug resistance makes sequencing one of the most versatile technology platforms for providing rapid, accurate and actionable results for treatment decisions. When the assay is designed correctly surveillance data can be a natural out follow. Currently there are no “plug-and-play”, “end-to-end”, sample acquisition-to-results interpretation NGS solutions for providing clinically-relevant sequence data for any disease in high income as well as LMICs. Nor are there commercialized solutions for sequencing the *Mycobacterium tuberculosis* complex (MTBC) genome from primary clinical samples (i.e. sputum). However, such a system-based solution has been envisioned and is under developed by a collaboration between academia, Non-Governmental Organizations and industry. The solution will be modular and is being designed and developed to perform targeted amplicon sequencing directly from a patient’s primary sputum sample. It is being designed as a solution addressing sample acquisition-to-data interpretation. This will allow, initially reference laboratories, to perform reflex NGS to provide a rapid and comprehensive analysis of a patients MTBC drug resistance profile, facilitating the optimization of treatment for the patient, reducing the spread of DR-TB and most importantly improved treatment outcomes. In addition, such a solution enables countries to implement culture-free surveillance programs bypassing the need for expensive culture facilities, decrease country dependence on external laboratories and significantly expand the map of global surveillance. The introduction of such a solution is a foundational employment of a key multi-functional platform which has the ability to empower LMICs in all areas of infectious diseases where NGS can provide a valid solution.

L 4

Update on free database

Stefan Niemann

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Global Priorities in TB research

Daniela Maria Cirillo

GL 2

Breakpoints for new and old anti TB drugs

Claudio Köser

GL 3

Tuberculosis: the impact of lesion diversity on drug penetration and sterilization

Veronique Dartois

GL 4

New drugs in action: case reports

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Ttreatment outcomes of multidrug-resistant (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) remain poor. In Belarus, only 54% MDR-TB and 36% XDR-TB patients starting treatment in 2014 completed it successfully. New medicines like bedaquiline (BDQ) and delamanid (DLM) are now being included in longer treatment regimens to increase treatment success. BDQ and DLM were introduced programmatically in Belarus in July 2015 and June 2016, respectively.
The Belarus TB programme developed measures to monitor safety and effectiveness of new containing regimens in line with the World Health Organization recommendations.
By May 1, 2017, 334 patients had started BDQ (n=297), DLM (n=33) and BDQ+DLM (n=4) containing regimens: 73% males; 42% previously untreated for TB; 64% XDR-TB; 18% with MDR-TB+FQ resistance and 13% MDR-TB+injectable resistance. Data on DLM treatment is still limited for analysis due to small number of patients and short period of treatment. By 6 months after starting BDQ treatment 198/208 patients (95%) had converted to culture negative (70% conversion at 2 months). Of 168 patients on BDQ followed up for at least 12 months: in 1 (< 1%) treatment failed, 3 (2%) died, 8 (5%) were lost to follow up, and 156 (92%) continue treatment with culture-negative sputum. All patients experienced adverse events, the most clinically relevant being abnormal liver function, hypokalaemia, hypomagnesaemia, hyperuricemia, cardiac arrhythmia (clinically insignificant), anaemia, low platelet count, lowered creatinine clearance, paraesthesia, and hearing loss. Most adverse events were of mild to moderate in severity and did not require BDQ withdrawal or stopping treatment.
Conclusions: Interim results show high culture conversion and safety of the BDQ containing regimens. Our patient series will help increase the global knowledge base for M/XDR-TB patients treated with new drugs containing regimens under programmatic conditions.

GL 5

Protocols for validating new tools for latent tuberculosis

Alberto Matteelli

GL 6

Towards signatures for TB progression

Hazel Dockrell
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To control tuberculosis, improved tools including new vaccines, diagnostic tests and better drugs are needed. To help with the selection of new TB vaccine candidates, biomarkers that would predict protection would be very valuable. Despite much effort, and the identification of essential cells and cytokines, it has been challenging to identify protective biosignatures, perhaps due to the complex nature of infection and disease caused by Mycobacterium tuberculosis. The GC6-74 consortium recruited HIV-negative

household contacts of newly diagnosed TB patients, and followed them over two years, in a multicentre study in Africa. Samples from those individuals who progressed to TB disease were compared with selected contacts who remained healthy, using RNAseq analysis. These results were used with those from a large study of South African adolescents to derive a "Correlate of Risk" biosignature that could predict the development of clinical disease 12-18 months before diagnosis (Zak et al Lancet 2016; 387:2312). Other approaches include using proteomics and metabolomics as well as functional assays such as mycobacterial growth inhibition. Although further work needs to be performed to derive point-of-care assays that would be useful in the clinic, tests to predict progression to TB would have huge public health benefit, as well as facilitating the trials of new TB vaccines.

This work was funded by the Bill and Melinda Gates Foundation through the GC6-74 Consortium and the GC6-2013 Consortium (#3772), and by the European Union Horizon2020 TBVAC2020 Consortium (#643381); the Adolescent Cohort Study was funded by the Bill and Melinda Gates Foundation (#OPP1021972 and OPP1023483)

GL 7

TB in children what is new from diagnosis to therapy

Steven Welch
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Childhood TB has long been neglected in public health policy, and has depended on data from adult studies to inform treatment. Globally, children represent 10% of the 10.4 million cases diagnosed in 2015, but disproportionately make up 12% of 1.8 million TB deaths. The preponderance of paucibacillary disease in children has always resulted in lower rates of bacteriological confirmation in children than adults. This in turn reduces the opportunity for drug susceptibility testing, and for genetic characterisation of the organism to aid in the understanding of transmission. Newer techniques aim both to improve sampling rates and sampling quality to maximise the rates of bacteriological confirmation, and to improve the accuracy of clinical diagnosis and other investigations when bacteriological confirmation is not possible.

Tuberculosis treatment guidelines based on adult data may get both the dosing and the duration of treatment for children wrong, and there has been very little data to inform treatment of drug-resistant TB in children. In 2017, clinical and pharmacological research in children is beginning to investigate more appropriate dosing regimens that may be more appropriate for both childhood pulmonary TB and TB meningitis. Access to drugs and provision of appropriate drug formulations remain important issues.

GL 8

Population structure and transmission of MTB

Sebastian Gagneux

GL 9

High-resolution analysis of *M. abscessus* and *M. chimaera* transmission dynamics and population structure by whole genome sequencing

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All mycobacterial species not causing tuberculosis or leprosy are referred to as nontuberculous mycobacteria (NTMs), a highly diverse group of more than 170 recognized species present in both environmental and human-associated habitats. Many NTMs are opportunistic human pathogens, especially under immunosuppression or chronic diseases such as cystic fibrosis, causing severe and treatment-resistant infections. Apart from individual acquisition from environmental sources, NTMs are also known for healthcare related outbreaks often linked with water reservoirs or distribution systems. Due to an increasing number of reports connecting NTM infection with human disease, several species have been recognized as important threats to public health. However, conclusive analysis of transmission dynamics and population structure has been hindered by the ubiquitous environmental presence of NTMs and the limited resolution power offered by traditional strain typing methods.

Here, next generation sequencing technology enables the rapid determination of near complete genomes, allowing for highest resolution genotyping and phylogenetic analysis. Recent studies using NGS could discern the *M. abscessus* overall population structure, delineate the emergence and global spread of three dominant clonal lineages associated with resistance and virulence, and present evidence for individual transmission chains by in-depth longitudinal characterization of patient subpopulations and

intra/inter-patient evolution.

Likewise, whole genome analysis of a healthcare related worldwide outbreak of *M. chimaera* could unravel the underlying transmission dynamic by demonstrating the global spread of individual strains including the co-colonization of hospital equipment by multiple strains.
Genome based analysis of known and emerging NTM pathogens is clearly a valuable tool to recognize and characterize present and emerging threats to public health, enabling targeted interventions to prevent transmission and improve treatment.

ABSTRACTS OF ORAL PRESENTATIONS (OP)

Research needs to achieve eradication of tuberculosis

OP 1

pncA mutations in Mycobacterium tuberculosis is a strong predictor of poor treatment success in the therapy of multidrug resistant tuberculosis

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Background: Pyrazinamide has proven its efficacy in treating tuberculosis, also multidrug resistant tuberculosis (MDR-TB). In most high MDR-TB burden areas, including China, phenotypic DST is generally not in place. Molecular diagnosis could be alternative for detecting PZA resistance and guiding MDR-TB treatment. This study aimed to evaluate the role of pncA mutation in predicting poor treatment outcome.
Methods: In a population-based retrospectively cohort study, we enrolled 354 consecutive patients with confirmed MDR-TB registered and admitted for treatment between 2010 and 2014. The treatment was followed up monthly within the 24-month course of standardized MDR-TB treatment in terms of clinical manifestation and sputum conversion. All isolates from subjects were tested for phenotypic susceptibility to PZA with the MGIT system, and mutations in the pncA gene were assessed by whole-gene sequencing.
Result: Among the 354 MDR-TB subject available for data analysis, 115 (32.5%) were phenotypically resistant to PZA, with 56 (48.7%) simultaneously resistant to a fluoroquinolone and/or aminoglycosides. Isolates from 106 MDR-TB patients had a mutation in the pncA gene or its promoter region, of which 102 (96.2%) were found to be phenotypically resistant to PZA. Among 304 MDR-TB subjects with known outcomes, treatment succeeded in 49.5% with initial resistance to PZA and 43.6% with genetic mutation in pncA, compared to 73.3% with plain MDR-TB and 67.7% with MDR-TB plus the resistance to fluoroquinolone and/or aminoglycosides. In further association study, adjusted by the identified confounders (Fluoroquinolone genotypic and phenotypic resistance, previous treatment history), PZA resistance was adversely associated with early sputum conversion and treatment success, while the drug resistance-associated genetic mutation in pncA even significantly increased time to sputum conversion and risk of treatment failure.
Conclusion: This report highlights the fact that sequencing of the pncA gene and its promotor can offer a rapid and reliable tool to predict the adverse treatment outcome in patients with MDR-TB.
Funding: NSFC program (No. 81373063); the joint NSFC-VR program (No. 81361138019)

OP 2

Evaluation of Deeplex-MycTB assay for the identification and antibiotic susceptibility profiling of Mycobacterium tuberculosis strains from primary specimens

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Background: Whole genome sequencing (WGS) holds the promise to revolutionize the diagnosis and epidemiological analysis of *Mycobacterium tuberculosis* complex (MTBC). However, its use in clinical practice is limited by the need to isolate the bacteria by culture. Alternative next generation sequencing (NGS) protocols providing clinical data from primary specimens have been recently developed. Among them the Deeplex-MycTB assay (Genoscreen), a multiplex PCR-based tool coupled to ultra-deep sequencing for rapid species identification, MTBC typing and resistance prediction. We evaluated the diagnostic performance of this kit on both indirect and direct samples.
Methods: A total of 187 sputum specimens and 35 isolates were tested by Deeplex-MycTB. Smear positive sputa collected during the national anti-TB drug resistance survey conducted in Djibouti in 2015 were sent to our laboratory using the sample transport reagent OMNIgene-SPUTUM

(DNA Genotek). DNA was extracted from one third of the sputum volume. Amplicons generated by Deeplex-MycTB were sequenced on Illumina MiniSeq platform. Sequence variant calling was performed using a dedicated software developed and pre-calibrated by Genoscreen. Results from targeted deep sequencing were compared to WGS data obtained from the corresponding isolates on 50% of samples, using a distinct variant calling pipeline.

Results: Of the primary specimens tested by Deeplex-MycTB, 182/187 (97.3%) provided reads aligning to MTBC and target genes. In particular, Deeplex-MycTB succeeded in producing sequences for all 21 targets in 160/187 (85.6%) of the samples with at least 5 or more reads aligned to all reference sequences while 22 (11.7%) samples had partial results with more than two targets not providing any exploitable data. Concordance between Deeplex®-MycTB and WGS performed on the corresponding isolate was 100% for rifampicin, fluoroquinolones and second-line injectables and 97.2% for pyrazinamide. The test missed one isoniazid resistant strain carrying a *katG* insertion outside the region targeted leading to an overall concordance of 97%.

Conclusions: Compared to WGS, Deeplex-MycTB showed high accuracy for rapid identification of MTB drug resistance-associated mutations directly from clinical specimens. This assay represents an important clinical tool for the optimization of patient's treatment and has the potential to significantly expand the capacity for drug resistance surveillance in countries without culture facilities.

OP 3

Widespread use of inappropriate MTBDRplus PCR conditions impacts performance

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MTBDR*plus*, a widely-used PCR test for multidrug-resistant tuberculosis (TB), has suboptimal sensitivities and indeterminate rates when done directly on smear-negative specimens. We investigated the impact of ramp rate usage (speed of temperature change between PCR cycles) on test performance.

Seventy-three laboratories using MTBDR*plus* were surveyed to assess the extent of incorrect thermocycler ramp rate usage. We also tested sputum from Xpert MTB/RIF-positive patients (52 smear-positive, 55 smear-negative) at the manufacturer-recommended ramp rate (2.2°C/s) and at an inappropriate ramp rate (4.0°C/s). Separately, dilution series of *Mycobacterium tuberculosis* strains were tested at 2.2°C/s and 4.0°C/s. *M. tuberculosis*-detection, indeterminate rates, accuracy, and inter-reader variability were assessed.

Of 23 respondent laboratories (each performing ~220 MTBDR*plus* assays per month), 78% used an incorrect ramp rate (with 4.0°C/s being the most common incorrect ramp rate). On smear-negative sputum, MTBDR*plus* was associated with improved TB detection (42/55 vs. 32/55 at 4.0°C/s; p=0.0422) and indeterminate rates (1/55 vs. 5/55; p=0.0931). Performance on smear-positive specimens were unaffected. In the dilution series, a ramp rate of 4.0°C/s but not 2.2°C/s resulted in false-positive rifampicin-resistant results at 10^2 CFU/ml, but not higher concentrations. At 4.0°C/s readers disagreed more about banding patterns.

Suboptimal performance of MTBDR*plus* on smear-negative specimens is likely partly due to use of an inappropriate PCR ramp rate, the use of which is widespread. Laboratories should ensure they use the correct ramp rate.

OP 4

What is resistance? Impact of molecular drug resistance testing on multi- and extensively drug-resistant tuberculosis therapy

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Rapid and accurate *Mycobacterium tuberculosis* drug-susceptibility testing (DST) is essential for the treatment of patients with multi- and extensively drug-resistant tuberculosis (M/XDR-TB). Discordant results from phenotypic and molecular assays represent a challenge and may impact on the design of individualised regimens.
Results of a) phenotypic DST (pDST) for 15 anti-TB drugs, b) whole genome sequencing (WGS) data for 33 resistance genes, and c) WHO-endorsed commercial molecular assays, i.e. Cepheid GeneXpert MTB/RIF (Xpert) and line probe assays (LPAs: Hain GenoType MTBDR*plus* 2.0 and MTBDR*sl* 2.0), were translated into individual algorithm-derived treatment regimens for 25 consecutive M/XDR-TB patients. Discrepancies between methods were assessed by minimum inhibitory concentrations (MICs) testing.
Beside high specificity of genotypic assays within their analytical range, mean overlaps of the inferred 'genotypic' regimens differed significantly compared to the pDST-derived therapy with just 49% (SD±24%) for Xpert and 63% (SD±16%) for LPAs and 93% (SD±11%) for WGS. Importantly WGS regimens did not comprise any drugs to which pDST showed resistance. Moreover, MIC-testing highlighted that pDST breakpoints for several key anti-TB drugs (rifampicin, ofloxacin, levofloxacin, moxifloxacin, kanamycin) were likely too high, and should be reconsidered.
WGS can be used to rule-in resistance even in M/XDR strains with complex resistance patterns, but pDST for some drugs is still needed to confirm susceptibility and construct the final regimens. But systematic misclassification of resistant isolates with particular mutations as susceptible due to pDST breakpoint artefacts, cause confusion for clinicians and may hamper the adoption of rapid genotypic assays that will be crucial for rapid and comprehensive molecular resistance predictions for individualized M/XDR therapies.

OP 5

A bespoke dry broth microdilution panel for susceptibility testing of M. tuberculosis: a validation study

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Background: CRyPTIC—a global effort to define the *M. tuberculosis* 'resistome' by whole genome sequencing up to 100.000 *M. tuberculosis* genomes—is underway. It aims to relate the effect of genomic mutations to the MIC of anti-tuberculosis drugs. As quantitative drug-susceptibility testing (DST) is currently non-routine and expensive when applied to multiple drugs, we have designed a bespoke, dry-form, 14-drug microtitre plate, containing multiple doubling dilutions for RIF, RFB, INH, EMB, MOX, LEV, KAN, AMI, LZD, CFZ, PAS, ETH, DLM, BDQ. Here we present a validation study.
Material/methods: A panel of 21 strains (H37Rv reference strain plus 20 phenotypically and genomically well-characterised EQC strains) was shared by the San Raffaele Scientific Institute, Milan, with 6 other participating centres. H37Rv was tested 10 times in each centre; 10 EQC strains were replicated at source (blinded), and all 20 (30 including replicates) EQC strains were again duplicated in each centre (unblinded). Inocula of 10^4 CFU/mL per isolate were obtained from solid media cultures, and automatically inoculated onto 96-wells microtitre plates. MICs were determined for each plate by 2 independent readers, using manual (mirrored-box and inverted-light microscope) and digital (Vizion™) viewing systems for inspection of growth. Readings took place at 4 different time points post-incubation (days 7, 10, 14 and 21) and were recorded on a web-enabled database.
Results: A total of 10302 plate observations were made. Preliminary analysis of results showed variance across replicates to be greater between centres than within centres, and greater across blinded replicates than unblinded replicates. 33% of observed MICs were either equal to or lower than the lowest assayed drug concentration, or greater than the highest concentration. The mean proportion of strain/drug observations that were within a single doubling dilution of the mode was 84% (SD 16%). PAS performed least well of all drugs. The optimal plate reading time was at 14 days by Vizion™. When compared to independent assay results, the microtitre plate

MICs were largely consistent with those obtained from the REMA and agar reference methods, whilst categorical agreement with MGIT DST was also encouraging.
Conclusions: Preliminary results are promising, and suggest this bespoke microtitre plate will become a valuable addition not only to the CRyPTIC effort, but to TB DST in general. Results indicate that reproducibility can be improved by further training, whilst an automated plate reading system is currently under development, which may also help address inter-operator variability. Further analysis is awaited to assess the need for any redesigning of the plate.

OP 6

BashTheBug.net: A citizen science project helping to classify M.tuberculosis drug susceptible tests

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The CRyPTIC project is an international consortium that between now and 2020 is collecting 100,000 TB samples at over a dozen centres. 40,000 samples will have their whole genome sequenced and their sensitivity to 15 different anti-tuberculosis (TB) drugs assessed, primarily through the use a specially-designed 96-well microtiter plate. Since the ultimate goal of the CRyPTIC project is to infer new resistance-conferring mutations in TB, it is important that the error in the phenotypic assessment by as small as possible. Hence, in addition to each plate being assessed by an expert in each centre, we have launched a Zooniverse.org citizen science project, called BashTheBug.net, which enlists members of the public to help us classify the plates.
The volunteers are shown the control wells from the plate which have no antibiotic present so they have an idea of what constitutes bacterial growth, then they are shown the series of wells for a single antibiotic (with the concentration doubling between successive wells) taken from the same plate. They are then asked which well is the first one in which bacteria do not grow. Each image is shown a minimum of 15 times with outliers being discarded, ensuring an accurate consensus is reached for each minimum inhibitory concentration.
BashTheBug was launched on 7 April 2017 - up to date statistics on the number of classifications performed can be obtained from http://bit.ly/bashthebug. However, during beta-testing alone over 600 volunteers made around 35,000 classifications. We shall describe the challenges of operating a project of this nature as well as compare the results to those of the experts and also an automated bacterial growth detection system. Ultimately, of course, these methods are not in competition, instead, we shall outline how they could best be used together to produce an optimal clinical microbiology workflow. For example, although it is unlikely a clinical microbiology workflow would involve inviting members of the public to help with bacterial growth classification, a similar web-based system could be used within a national reference microbiology service to gain a consensus on especially difficult cases.

OP 7

The correlation of phenotypic and genotypic resistance of Mycobacterium tuberculosis on different levels of drug concentration

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Introduction: Rapid identification of drug resistance pattern of Mycobacterium tuberculosis (MTB) allows an earlier initiation of an adequate treatment regimen that potentially can reduce TB morbidity, mortality and transmission. New diagnostic methods have provided a promising solution for rapid and reliable detection of drug resistant TB strains. Despite the fact that rapid molecular assays are less accurate than the culture based methods and raise the possibility of false negative results, the molecular characterization of the resistance spectrum of MTB isolates offers the opportunity for overcoming the phenotypically detected resistance. So far, mutations within the rpoB gene confers a different level of phenotypic resistance for rifampicine (RIF) as well as mutation in inhA is link with low resistance to isoniazide (INH).
Aim: To study the correlation between phenotypic and genotypic resistance of MTB on different drug concentration levels.
Methods: The genotypic resistance profiles for isoniazide and rifampicine of MTB sputum

isolates were assessed by MTBDRplus v.2 and where correlated with culture based (Bactec MGIT 960) drug sensitivity test results. The different level of inhibitory concentrations of rifampicine and isoniazide of individual strains, assessed by Bactec MGIT 960 equipped with EpiCenter TB eXiST, were correlated correspondingly with the mutation types in the rpoB gene, and the presence of inhA mutation in the same MTB isolates.

Results: The sputum MTB isolates from 4568 patients with pulmonary tuberculosis (TB) were assessed. 64.2% of them were INH resistant but only in 1,9% (n=86) of the isolates, inhA mutation were identified. RIF resistance were detected in 61.9% of subjects, and in 27.2% (n=762) RIF resistance was associated with other mutations than S351. 30.6% of INH resistant MTB strains, conferred by inhA mutation only and 28.6% of RIF resistant MTB strains without S351 mutation, were sensitive to high concentrations of drugs by phenotypic DST.

Conclusion: The correlation of genotypic tests results with phenotypic resistance levels can be crucial forward a personalized approach in TB patient treatment, stopping the spread of drug resistance and promotion of the optimum use of the few drugs available for resistant TB treatment.

OP 8

Evaluation of Abbott RealTime MTB INH/RIF Resistance Assay using well-characterized M. tuberculosis Isolates from Central Asia

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Worldwide, one third of all tuberculosis (TB) cases and almost three quarters of the 480'000 cases with multi-drug resistant (MDR) TB, defined as resistance toward rifampicin (RIF) and isoniazid (INH), are not notified. Molecular detection of genetic resistance markers directly in clinical specimen is playing a pivotal role in early notification of resistant TB cases thereby allowing rapid interruption of transmission chains by adequate isolation and treatment of patients.

We have recently evaluated the Abbott RealTi*me* MTB and MTB INH/RIF assays, real time PCR systems for high-throughput diagnostics of TB including resistance markers for INH and RIF. The resistance markers identified by MTB INH/RIF assay were found to be in nearly full concordance with those from GenoType MTBDR*plus* and phenotypic drug susceptibility testing. A limitation of this previous study, however, was the low number of resistant samples tested. In this extended follow-on study we aimed to assess the full capacity of MTB INH/RIF assay by testing pre-characterized clinical isolates with a broader spectrum of resistance patterns and *rpoB*, *katG*, and *inhA* mutations.

We analysed 339 clinical *M. tuberculosis* isolates recovered in the frame of national drug resistance surveys in Central Asia by MTB INH/RIF assay. For this purpose, DNA was extracted from inactivated culture samples on the Abbott *m*2000*sp* platform, transferred to MTB INH/RIF test plates and analysed with the *m*2000*rt* real time PCR instrument. Results were compared to those from GenoType MTBDR*plus*, Sanger sequencing of the *rpoB* gene, and phenotypic susceptibility testing. INH resistance markers showed high concordance (>99%), whereas few discordances were observed with RIF resistance markers. Those were either associated with heteroresistance, i.e. the co-existence of wild-type and mutation *rpoB* signals in Genotype MTBDR*plus*, or with the presence of disputed *rpoB* mutations at positions 526, 527, or 533. Additional discrepancies with resistance phenotypes were only observed with *rpoB* mutations outside the rifampicin resistance determining region. The full data including gene sequencing and the minimal inhibitory concentrations of INH/RIF are currently under final analysis and will be reported in the planned presentation.

OP 10

Personalized treatment for tuberculosis: lessons from a single case over eight years of disease

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Personalized treatment in tuberculosis can be achieved in the next years if we are able to implement rapid, cost-effective and comprehensive drug susceptibility tests. The role of whole genome sequencing will become particularly relevant if it can avoid the limitations of the current gold-standards, i.e. culture methods and nucleic acid amplification tests. By whole genome sequencing we have been able to diagnose a multidrug resistant strain infecting a patient over the course of more than eight years

and for which routine diagnostic algorithms did not detect rifampicin and isoniazid resistance. Using whole genome sequencing in retrospective and prospective manner we aid in the clinical management of the case in the absence of reliable DST results. In addition, by combining whole genome sequencing with amplicon-based sequencing and clone analysis we are able to 1. identify new genetic determinants of isoniazid resistance co-existing in the patient; 2. show unexpected highly dynamic nature of drug resistance to first-line antibiotics over eight years and 3. use the genome information to trace the direction and timing of transmission to a household contact. In summary, we show that whole genome sequencing can become a primary diagnostic approach and can be used to guide treatment of tuberculosis infection. Furthermore, in some exceptional cases like the one presented it can be the sole reliable diagnostic approach. However, we also show the many challenges ahead including the validation of low and extremely-low heteroresistance variants detected and the interpretation of these variants for clinical decision making.

OP 11

Combination of antimicrobial peptides and isoniazid/rifampicin against multidrug-resistant tuberculosis

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Multidrug-resistant (MDR) *Mycobacterium tuberculosis* (Mtb) bearing resistance towards both isoniazid (INH) and rifampicin (RIF) were reported during early 1990s. New anti-TB drug development has been extensively focused, yet the adverse events experienced by patients were substantial. Therefore, innovative approaches to achieve a shortened and safe therapeutic regimen for drug-resistant TB is imperative.
The specific composition of mycobacterial cell wall giving rise to its extremely high hydrophobicity and impermeability is known to confer *Mtb* antibiotic resistance. Antimicrobial peptides (AMPs) act selectively on negatively charged bacterial membranes, their pore-forming actions lead to leakage of cellular content and eventually cell death. This killing effect is rapid and potent while making development of resistance to AMPs difficult. Our study aims at investigating the combination of novel antimicrobial peptides with first line anti-TB drugs against MDR-TB. D-LAK peptides were shown to exert a detergent-like effect which effectively broke down clumps of bacteria and inhibited the growth of MDR and XDR Mtb strains (1). Therefore, we hypothesize that D-LAK peptides can facilitate the access of anti-TB drugs into mycobacteria by increasing their surface permeability, with the potential of re-sensitizing MDR-TB to first-line anti-TB drugs. Combination treatment of INH and RIF with D-LAK peptides has successfully demonstrated synergistic effect against the growth of MDR-TB strains. Antibacterial assays revealed the efficacies of bacterial load reduction at a lower anti-TB drug as well as D-LAK peptide concentrations. We have also employed *Mycobacterium smegmatis* as a model to understand the mechanism of actions of D-LAK peptides. Membrane disruption activity by D-LAK peptides was visualized using transmission electron microscopy (TEM). Prolonged treatment of peptide caused irreversible damage of cell envelope leading to leakage of cellular content. Confocal microscopy has further confirmed cell surface perturbation action of D-LAK peptides supporting our hypothesis regarding the assistant role of peptides in re-sensitization of MDR-TB towards first-line drugs. Further investigations on the antibacterial mode of action in a whole organism view using nuclear magnetic resonance (NMR) metabolomics is in progress.

1. Lan Y, Lam JT, Siu GK, Yam WC, Mason AJ, Lam JK. Cationic amphipathic D-enantiomeric antimicrobial peptides with in vitro and ex vivo activity against drug-resistant Mycobacterium tuberculosis. Tuberculosis (Edinb). 2014;94(6):678-89.

OP 12

Evaluation of whole genome sequencing for drug susceptibility testing of Mycobacterium tuberculosis

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The emergence of multi-drug resistant tuberculosis (MDR-TB) is a major public health concern, with an estimated global prevalence of 5% and large geographical variation. Culture-based assays are currently the gold standard for drug susceptibility testing and provide good sensitivity and specificity but are time-consuming. The objective of this study is to evaluate whether whole genome sequencing (WGS) can replace routine culture based assays for drug susceptibility testing to reduce the time needed to advice on optimal drug treatment to the patient. Furthermore, we evaluated the laboratory costs of WGS compared to routine culture-based assays. All *Mycobacterium tuberculosis* (MTB) cultures sent to the national reference laboratory in 2014 (n=213) were phenotypically tested by mycobacteria growth indicator tube (MGIT) as gold standard for first line drugs and streptomycin. WGS was performed on all MTB isolates using the Illumina MiSeq platform and data were analysed using the PhyResSE online analysis tool. Three samples could not be sequenced due to a low DNA concentration and the median coverage among remaining n=210 samples was 72 (range: 8-119). Overall, 189 of 190 (99.5%) susceptible isolates were correctly predicted by WGS, and 16 of 20 (80.0%) resistant isolates were predicted as containing at least one resistant marker. The sensitivity of isoniazid (H) and rifampicin (R) was 81% (CI: 54.4-96.0%) and 100% (CI: 63.0-100%), respectively, and specificity 100% (CI: 98.1-100%) for both drugs. The sensitivity of ethambutol (E), pyrazinamide (Z), and streptomycin (S) were 0% (CI: 0-97.8%), 40.0% (CI: 5.3-85.3%), and 91.7% (CI: 61.5-99.8%), respectively. The specificity of drugs E, Z, and S ranged from 97.6-100% (max. CI: 94.5-100%). The reagent costs for WGS were ~5 times higher compared to MGIT. In conclusion, WGS has a high specificity for all five assessed drugs, but lacks sensitivity to predict drug resistance for some drugs, especially E and Z, which are known to be problematic drugs in culture based assays as well. WGS could be used as a pre-screening assay to identify MDR strains (resistant to H and R) with high sensitivity and specificity and reduce the time spent in the BSL3 laboratory and diagnostic time to one week or less for the majority of specimens. Phenotypic assays are currently still needed to confirm diagnosis and to determine drug resistance among MDR and XDR specimens

OP 13

Use of Whole Genome Sequencing approach for diagnosis of drug-resistant tuberculosis in a routine setting

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Whole Genome Sequencing (WGS) allows «all-in one» diagnosis of drug-resistant tuberculosis (DR-TB) providing results on species identification and typing, drug susceptibility patterns and transmission events. This method is suitable on early positive cultures, enabling to quickly start treatment of TB patients and guide public health interventions, to save time and costs compared to standard phenotypic testing, and to provide more information than the WHO-endorsed molecular assays. Aim of this study was to evaluate the feasibility to introduce WGS in routine TB diagnosis workflow with a centralized approach.

We processed by WGS 175 BD BACTEC MGIT 960 positive isolates referred by the TB Regional Reference Center of Tuscany in a 1-year time frame. DNA was isolated from 2 mL aliquots of heat-inactivated early positive cultures by using the Promega Maxwell 16 MDx Extraction System. Paired-end libraries were prepared using the Illumina Nextera XT DNA Sample Preparation kit, and sequenced on Illumina Miseq, MiniSeq or HiSeq 2500 platforms. Total variant calling was performed by using a dedicated pipeline. WGS turnaround time and accuracy were compared with the standard testing performed in the routine laboratory.

Time to availability of WGS report was around 72 h from culture positivity compared to average 3 weeks for conventional MDR-TB diagnosis, at cost per strain of approximately € 150 inclusive of staff, reagents and instruments. A cut-off of >30x mean coverage and >90% mapped reads was used to define high-quality WGS results. In our dataset, results were optimal for 93 samples and acceptable for 62 cases (>15x mean coverage).

For the remaining 20 samples, sequencing results would need to be confirmed by conventional testing since reads coverage was <15x (mapping <90%): main challenge was the presence of non–mycobacterial DNA contamination in variable amount. We observed high diagnostic accuracy for species identification and for detection of full DR profile compared to phenotypic testing, with the advantage to provide early evidence for 2nd-line resistance, 3 confirmed TB outbreaks and 4 laboratory cross-contamination events.
The implementation of WGS in routine laboratories would allow to significantly shorten time and costs to TB diagnosis. Importantly, its use at centralized level in a low TB incidence Country would drive epidemiological investigation of spreading DR-TB cases in «real-time», promptly informing the Ministry of Health agencies.

OP 15

Single-live imaging system for the analysis of in vitro/ex vivo macrophage infection models for tuberculosis

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One of the main features of *M. tuberculosis* (Mtb) is its ability to block the phagolysosome maturation during macrophage infection. We planned to compare phagolysosome induction by different clinical isolates belonging to different lineages during THP-1 derived macrophage-like cells and human monocyte-derived macrophages (MDMs) infection. We also set up a single-live cell imaging system based on the CellASIC ONIX platform (Merck Millipore) to consider the variability at individual-cell level, usually underestimated by population averaged studies.
We compared LysoSensor Green (ThermoFisher) signal from lysosomes of THP-1 and MDMs infected with different lineages (Africanum – L6, Beijing – L2, and H37Rv – L4) during confocal microscopy live-imaging. We adapted the CellASIC ONIX system to be used in live-imaging macrophage infection models using *M. bovis* BCG. The microfluidic plate allows continuous flow of solutions from the inlets to the four cell culture chambers, generating a dynamic exposure profile during live-cell imaging. The plate houses all experiment solutions allowing to control the flow rates with an external pneumatic manifold without perturbing the microscope stage.
Cells infected with H37Rv were characterized by a lower number of lysosomes and this difference was found to be statistically relevant in THP-1 ($p\text{-val}<0.05$). Despite not statistically significant, the same results were obtained in the MDM infection model. Considering LysoSensor intensities (proportional to acidic pH), in MDMs we observed higher values during infection with Africanum and Beijing, compared with H37Rv. This trend was reversed in THP-1 infection model where infection lead to a reduction in the signal from lysosomes, and H37Rv infection lead the highest intensities. In adapting our protocols to the microfluidics, we found that a low pressure-/gravity-driven mixed approach for loading plates is required to maintain healthy cells. Proper polarization of macrophages is required to increase mycobacterial uptake during the infection protocol in the microfluidic environment.
Our data support the hypothesis that isolates belonging to different lineages causes different responses within macrophages, highlighting relevant differences between laboratory strains and clinical isolates. The single-live cell imaging system CellASIC ONIX can be used for *in vitro/ ex vivo* models infection and will further tested for monitoring of apoptosis/necrosis, autophagy, and ROS/NO production.

OP 16

Genetic and functional analysis of polymorphisms in the PKLR gene and association with leprosy and tuberculosis

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PKLR, polymorphisms, leprosy, tuberculosis, iron
Leprosy and tuberculosis (TB) are chronic infectious diseases in which both host genes and environmental factors play a role in disease outcome. *PKLR* encodes for pyruvate kinase (PK), a key red blood cells enzyme that catalyzes the ATP output in the glycolytic system. This gene is under a strong selective pressure by pathogens in Africa where malaria is endemic. Mutations responsible for PK deficiency have a dual role: association with resistance to malaria and susceptibility to *Salmonella*

typhimurium. Thus, aiming to investigate the *PKLR* polymorphisms and its association with mycobacterial pathogens, we performed two independent case-control studies with leprosy and tuberculosis in Rio de Janeiro and a Mozambique population, respectively. Our results highlighted the haplotype G/G/G/G (rs11264355, rs4620533, rs4971072 and rs11264359) as associated with leprosy susceptibility in individuals from Rio de Janeiro (frequency in cases – 0.39 – and controls – 0.27). Noteworthy, *PKLR* SNPs rs4971072/ rs11264359 captured most of the information while haplotype G/G was strongly associated with risk to leprosy in Rio de Janeiro (OR=1.86, p<0.00001). Subsequently, association was confirmed for two SNPs (rs11264355-G allele and rs11264359-G alelle) in an independent TB case-control Mozambican population. Also, the frequency of haplotype G/G/G/G was higher in cases (0.48) than in controls (0.38), which suggests the same direction of association in Mozambique. The increased G/G/G/G frequency in Mozambicans indicated ancestry footprints of African origin among Brazilians. In fact, comparison of haplotype frequencies in different populations from the EPIGEN Consortium and 1000Genomes showed that the associated haplotype G/G (rs4971072/rs11264359) is augmented in Salvador (0.56) and African population (0.87) compared to Pelotas (0.32) and European ancestry cohort (0.27). Then, we evaluated the genotype-phenotype correlation by comparing iron parameters within the *PKLR* SNPs. We observed that GG-genotype (for all individual SNPs) correlates to higher ferritin and haptoglobin load within individuals, which supports a relevance role of the *PKLR* gene in mycobacterial infections.

Support: CNPq and CAPES.

OP 17

Contribution of efflux pumps to Rifampicin resistance in clinical isolates of M. tuberculosis

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Despite the consideration of chromosomal mutations as the major cause of rifampicin (RIF) resistance in *M. tuberculosis*, the role of other mechanisms such as efflux pumps cannot be ruled out. We evaluated the role of four efflux pumps *viz., Rv0507*, *Rv0676c, Rv0194* and *Rv1250* in providing RIF resistance in *M. tuberculosis*. The real time expression of the efflux pumps was analyzed in 17 RIF resistant and 10 RIF susceptible clinical isolates of *M. tuberculosis* after exposure to RIF. Expression of efflux pumps in these isolates was also correlated with mutations in the *rpoB* gene and MICs of RIF in the presence and absence of efflux pump inhibitors. Under RIF stress, *Rv0676c* was induced in 7/17 (41%) RIF resistant and 1/5 (20%) RIF susceptible isolates; *Rv0194* was induced in 9/17 (53%) RIF resistant and 1/5 (20%) RIF susceptible isolates; *Rv1250* in 5/17 (29%) RIF resistant and 1/5 (20%) RIF susceptible isolates; and *Rv0507* was upregulated in 2/17 (12%) RIF resistant and 1/5 (20%) RIF susceptible isolates. Alterations in MIC of RIF in the presence of efflux pump inhibitors verapamil and CCCP were studied in a subset of isolates (5 RIF susceptible and 7 RIF resistant) and revealed 4-64 fold reduction in both RIF resistant and RIF susceptible isolates. This study, for the first time, suggests an association of *Rv0676c* with RIF resistance in *M. tuberculosis*. Extensive studies are needed to determine the role of efflux pump inhibitors as adjunct therapy in tuberculosis.

OP 18

HIV and tuberculosis in an intermediate-incidence country – a retrospective study at a Portuguese Infectious Diseases Department

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Introduction: Tuberculosis (TB), an infectious disease caused by *Mycobacterium tuberculosis*, is one of the oldest diseases in history and a public health threat (1). Despite the steadily decline in TB number of cases, Portugal is still an intermediate-incidence country, the only one in Western Europe (2, 3). HIV incidence was 9.5/100 000 population in 2015 and HIV patients represented 14% of all TB cases diagnosed (2, 3).

Portugal's drug policy and approach to integrated care (TB/HIV and drug dependency care) is

distinct from many other parts of Europe (4).
Methods: We performed an observational retrospective study. We evaluated all patients with HIV and TB co-infection admitted to the Infectious Diseases department of São João Hospital Center, Portugal, between 2008 and 2014.
Results: For the studied period, 139 patients were admitted to hospital with the diagnosis of TB (13% of hospitalized patients with HIV) and in 37% TB was the first manifestation of HIV infection. Seven percent were patients from areas of high TB endemicity, 45% had a precarious social situation and 47% were active or former drug users. CD4 lymphocyte count was below 200/mm3 in 77% and 35% were highly immunodepressed (CD4 lower than 50/mm3). Of those with previously known HIV infection, 28% complied to HAART with good adherence, only 25% (n=22) had been screened for latent tuberculosis, and of these, 1 had positive screening and received latent TB treatment. Regarding TB, 57% had a definitive diagnosis, 34% possible and 9% probable. Disseminated TB was present in 33% of patients and 41% had concomitant infections. Mean time between onset of symptoms and initiation of treatment was ~47 days, with no statistically significant differences between patients with and without precarious social situations (p = 0.076). Of the 99% of patients who started TB treatment, 26% experienced toxicities and 64% completed treatment.
Conclusion: Tuberculosis is a major opportunistic infection in HIV patients, especially in immunosupressed ones with poor adherence to HAART. Although a precarious social situation is a risk factor for TB, in our study this didn't lead to delayed diagnosis or treatment, nor to worse prognosis. Of notice, latent TB screening was performed in a minority of patients, highlighting the need to scale up diagnosis and treatment of latent TB in HIV population.

OP 19

Differential No-PDIM Lipid Production in rifampicin-resistant and H37Rv Mycobacterium tuberculosis strains: search of rifampicin resistant Biomarkers

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Introduction: Multidrug-resistant tuberculosis (MDR-TB, rifampin and isoniazid - resistant *Mycobacterium tuberculosis* infection) is burgeoning in emerging countries. New and efficacious therapeutic, diagnostic and prognostic alternatives are needed. There is an imperative for potential antibiotic-resistant infection biomarkers and correlating this with the levels of bacterial products instead of classical immunological biomarkers. This work proposed to analyze by shotgun lipidomics differences between Rif – sensible controls and a Rif-R strain in addition to the already described PDIM excess production in RifR strains.
Methodology: It was designed a cell-wall lipid extraction procedure in solid (Löwenstein-Jensen) and liquid cultures (MGIT) and lipid mass spectrometry analysis using the reference strain H37Rv and 2 clinical strains (UN-B077bc susceptible control and Rif-resistant (Rif-R) INS-2420/21). Analytic PDIM standard purified from MTB H37Rv (Bei resources) was used for PDIM analysis. Samples from liquid broth were pre-concentrated using Florisil Solid Phase Extraction (SPE).
Results: SPE pre-concentration had a low recuperation percentage (13,45%) after toluene: heptane treatment. Using HPLC – qTOF analysis of solid culture extracts, when comparing with MS-LAMP database showed peaks at 22 – 24 minutes in RifR strains that were absent from both sensible H37Rv and clinical strains: we identified alpha, methoxy and keto mycolates (22 minutes of elusion), Glucose monomycolate (GMM, 22-23 and specially 24 min) and PIMs (Ac_1PIM_1 17 min, PIM_3 23 min, $LysoPIM_5$ 24 min). PDIM was found in all 3 solid medium samples but it was only found in control strain and standard but not in clinical isolated samples when cultured in broth. Clear separation of different PDIM fractions were obtained by using a simplified ammonium formate 10 mM pH 5,0 aqueous mobile phase.
Conclusions: We found some lipids in 17 and 22-24 minute elusion that were differentially expressed in Rif-resistant strain in comparison with 2 susceptible strains. These results could be used for lipid panels intended to distinguish Rif-resistant strains from Rif-susceptible ones.

OP 20

Association between interleukin-2, interleukin-4 gene polymorphisms and pulmonary multi-drugresistant tuberculosis

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Background and objective: To study the association between *IL-2, IL-4* gene polymorphisms and pulmonary multi-drug resistant tuberculosis (MDR TB).
Methods: The study comprised 170 individuals in Kharkiv region of Ukraine including 74 patients MDR TB (1st group), 66 patients without – MDR TB (2nd group) and 30 healthy donors (3nd group). Serum levels of cytokines IL-2 and IL-4 were evaluated by ELISA. Measurements on serum samples of patients were conducted prior or during first days after admission to the hospital. Investigations of gene polymorphisms of these cytokines were performed using restriction analysis of the amplification products of specific regions of the genome. Two polymorphic variants were examined: T-330G promoter region of *IL-2* and C-589T–*IL-4* genes.
Results: In the 1st group the levels of IL-4 and IL-2 were 7.96±0.29 pg/L and 39.34±1.14 pg/L, 2nd – 11.29±0.35 pg/L and 36.20±0.89 pg/L while in 3rd group these values were 29.99 ±1.27 pg/L and 21.60±0.80 pg/L respectively ($p<0.05$ between the groups). In patients with MDR TB the heterozygous genotype (79.73±4.67% (N=59) for *IL-2* and 71.62±5.24% (N=53) for *IL-4* genes) was higher than: 10.81±3.61% (N=8) and 14.86±4.14% (N=11) of patients had mutation genotype and normal homozygote genotype had 9.46±3.40% (N=7) and 13.51±3.97% (N=10) for *IL-2* and *IL-4* genes, respectively. In patients 2nd group the mutation homozygote genotype (65.15±5.87% (N=43) for *IL-2* gene and 69.70±5.66% (N=46) for *IL-4* gene) was higher than: 19.70±4.90% (N=13) and 13.64±4.22% (N=9) of patients had homozygous and normal homozygote genotype had 15.15±4.41% (N=10) and 16.67±4.59% (N=11) for *IL-2* and *IL-4* genes, respectively. In contrast, most of healthy donors had normal homozygous genotype with 60.00±8.94% (N=18) and low frequency of mutation genotype at 16.67±6.80% (N=5) and 23.34±7.72% (N=7) and heterozygous genotype at 23.33±7.72% (N=7) and 16.67±6.80% (N=5) for *IL-2* and *IL-4* genes, respectively ($p<0.05$ between the groups).
Conclusion: Compared to healthy controls patients with tuberculosis had significantly lower levels of serum IL-4 and high - IL-2. This coincided with greater frequency of heterozygous genotype in 1st group and mutation homozygote genotype in 2nd group polymorphisms C-589T and T-330G genes of *IL-4* and *IL-2*. Further studies are warranted whether higher rate of MDR TB has a causal immunogenetic relationship to polymorphism of genes encoding for *IL-2* and *IL-4* than patients without MDR TB. In addition, these studies revealed a significant influence of the polymorphisms genes *IL-2* and *IL-4* on the changes in the population of Th-lymphocytes, clinical symptoms, relapse of tuberculosis, formation destructions in the lung, which may treatment outcomes in patients with MDR TB.

OP 21

Comprehensive genome-wide analysis of Mycobacterium tuberculosis strains isolated from patients with tuberculous spondylitis

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Background: Genome-wide analysis of *Mycobacterium tuberculosis* strains isolated from patients with extrapulmonary tuberculosis, is interesting from medical and biological points of view. Here we report results of whole genome sequencing of *M. tuberculosis* isolates obtained from patients with tuberculous spondylitis from across Russia. These were all assessed for phylogenetic and strain placement, for biological drug resistance, for presence of known sequence substitutions associated with TB drug resistance, and for novel sequence variants, insertions, and deletions in coding regions of *Mycobacterium tuberculosis* coding genes.
Material/methods: Genomic DNA of 71 *M. tuberculosis* strains collected in 32 different regions of the Russian Federation in 2012-2015, was sequenced using Illumina MiSeq platform. *M. tuberculosis* H37Rv reference genome (NC_000962.3) was used for SNP and Indel calling using bowtie2, samtools and

FreeBayes tools. RaxML package was used for phylogenetic analysis, *Mycobacterium canettii* genome was used as outgroup. SpoTyping and PhyTB tools were used for classification of *M. tuberculosis* strains. Conventional spoligotyping was performed for 20 isolates. R-package was used for statistical analysis.

Results: Most isolates (n=58, 81.7%) belonged to Beijing genetic family predominating in Russia, remaining isolates belonged to LAM, Ural and two clades of T-family. Previously described mutations linked to streptomycin, isoniazid, rifampicin, pyrazinamide ethionamide and ofloxacin resistance were detected in drug resistant isolates. Bioinformatic analysis allowed to identify 29 deletions and 20 insertions (1-11 bp) tentatively associated with *M. tuberculosis* genetic clades identified in this study.

Conclusions: Whole genome sequencing of *M. tuberculosis* strains isolated from patients with extrapulmonary TB allowed identification known and new candidate gene markers useful for identification of genetic groups and offer a plausible explanation for Beijing genetic family pathogenesis and development of drug resistance.

Acknowledgements: This work was supported by Russian Foundation for Basic Research Grant 16-34-60163 and St. Petersburg State University Grant1.38.253.2015

OP 22

Unravelling the transcriptome of phenotypically different isogenic cells

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Background: Divers microenvironments are created within the host to which *Mycobacterium tuberculosis* need to adapt during infection. Therefore, metabolically and phenotypically heterogeneous populations are found in clinical samples which affect treatment outcome. Heterogeneous studies suggest that the efficacy of anti-tuberculosis drugs and the success of treatment outcomes is not only dependent on the model being used/studied, but also on the microenvironmental conditions (i.e. oxygen, nutrients, pH, cellular composition) within the different lesion types. We therefore hypothesis that phenotypically divers isogenic cells isolated from a single sputum sample have variable genomes and transcriptomes.

Methods: single bacilli were isolated from a heterogeneous population using a easy-to-use method established within our department for heterogeneous samples. After MICs were determined for the bacilli, DNA was extracted for whole genome sequencing on the Ion Proton platform. RNA was extracted and sequenced using the Illumina platform.

Results: Single bacilli with a low (40 µg/ml) and high (150 µg/ml) rifampicin MIC having the same Ser531Leu mutation was isolated from a heterogeneous sample.

In the absence of rifampicin: WGS comparisons identified sequence variations in regulatory proteins and well as phosphate transport in the low MIC strains. Suggesting that the low MIC strains have certain deficiencies in sensing and responding to the presence of rifampicin. RNA-seq analysis showed that the low MIC strains were sensing low intracellular concentrations of phosphate and in response up-regulated the expression of genes involved in phosphate transport to increase the intracellular concentration of phosphate.

In the presence of rifampicin: RNA-seq analysis identified a transcriptional signature induced by exposure to rifampicin irrespective of the genetic background. This signature comprised over expression of 13 genes, including 3 operons. Accordingly, we hypothesise that the bacilli are sensing the presence of the rifampicin and are responding by activating expression of these genes to change the porosity of the cell to restrict entry of rifampicin, to actively pump out rifampicin and to metabolise rifampicin.

Conclusion: The complex transcriptional response occurring in the presence of rifampicin suggesting that off-target effect of the drug exist, providing novel insights into the mechanisms whereby *M. tuberculosis* adjusts it physiology in response to certain compounds. This understanding may be key to the development of novel adjuncts to enhance the activity of anti-TB drugs.

OP 23

Population Structure and Clinical Presentation of Pediatric Mycobacterium tuberculosis in Florida, 2009-2015

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Background: Children are especially vulnerable to *Mycobacterium tuberculosis* infection. In low incidence settings, pediatric tuberculosis (TB) may serve a proxy for *M. tuberculosis* transmission. In this analysis, we describe the epidemiologic and clinical characteristics of TB in children 0-15 years and examine the *M. tuberculosis* population structure among 24-locus MIRU-VNTR genotyped cases.
Methods: This is a cross-sectional analysis of pediatric TB cases reported in the State of Florida from 2009 to 2015. We examined the sociodemographic and clinical characteristics of the cases. Where genotyping information was available, we analyzed the *M. tuberculosis* population structure using the web application MIRU-VNTRp*lus*.
Results: 4,871 TB cases were reported during the study period and 235 (4.8%) were pediatric cases. The children were predominantly male (51.9%) and African-American (44.3%). The median age at diagnosis was 4.0 years. While 75.3% were born in the U.S., 68.5% had at least one foreign-born guardian. A quarter was initially evaluated for TB as a close contact to a case and 26.0% due to TB symptoms. The majority presented with strictly pulmonary disease (76.2%). Extra-pulmonary presentation included meningeal TB (3.4%), TB of the bone and/or joint (2.1%) and lymphatic TB (13.6%). TB disease was confirmed by culture in 64 (27.2%) of the children and 56 (87.5%) had genotyping information available. Euro-American (75.0%), East Asian (7.1%), East African Indian (5.4%), and Indo-Oceanic (8.9%) were the predominantly *M. tuberculosis* global lineages. At the sub-lineage level, Haarlem 16 (28.6%), LAM 9 (16.1%), EAI 4 (7.1%), and Beijing 4 (7.1%) were the predominant strain families.
Conclusion: The bulk of pediatric TB cases in Florida over the past seven years resulted from *M. tuberculosis* transmission that occurred within the U.S.

OP 24

Spatiotemporal analysis of animal-adapted members of Mycobacterium tuberculosis complex: epidemiology connections revealed by fine-scale genome analyses

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Animal tuberculosis (TB), caused by *Mycobacterium bovis* (*M. bovis*), and less frequently *Mycobacterium caprae* (*M. caprae*), is a worldwide disseminated chronic infection that affects livestock and several wildlife species. This disease has a huge relevance in animal health and on animal trade/ animal-derived products-based economies.
In Portugal, animal TB is maintained in a multi-host pathogen system, with *M. bovis* circulating among wild hosts (wild boar and red deer) and livestock. Currently, a nationwide control and eradication program is implemented in the cattle population and a risk area for TB in red deer and wild boar is also established since 2011. However, the epidemiological situation is still far from the officially TB-free status, so a deeper knowledge of *M. bovis* transmission dynamics and resilience processes is key to design new interventions.
In this work, we set out to systematically characterize *M. bovis* and *M. caprae* isolates from long-term surveillance (2002–2015) in Portugal, with the objective of refining knowledge of pathogen population structure, reconstructing

the spatiotemporal history and disclosing the ecological processes underlying host shifts and host adaptation.
Standard molecular methods, as spoligotyping and MIRU-VNTR (*Mycobacterial Interspersed Repetitive Units-Variable Number of Tandem Repeats*), were applied to a wide panel of *M. bovis* isolates, obtained from tissue samples of cattle (*Bos taurus*) (n = 173), wild boar (*Sus scrofa*) (n = 132) and red deer (*Cervus elaphus*) (n = 145), from TB hotspot areas in mainland Portugal. Spoligotyping revealed more than 40 patterns, being SB0121, SB1174 and SB0119, the most frequent. Moreover, MIRU-VNTR analysis enabled the establishment of subtypes within the *M. bovis* isolates grouped by spoligotype. These techniques established the basis for the assessment of intra- and inter-specific genotypes. However, inferring chains of transmission and understanding the ecological role of each host in disease maintenance requires a genomic discrimination of the isolates at a finer scale, which will potentially be enabled by whole genome sequence analyses that has just been launched for a group of selected isolates.
The data obtained thus far reinforce the complexity of *M. bovis* epidemiology and emphasize the need of population-based studies based on progressively discriminatory techniques to clarify the peculiarities of infection by *M. bovis*.

OP 25

Time-and-Motion tool for assessment of working time in tuberculosis laboratories: a multi centre study

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Timely and accurate diagnosis of active tuberculosis (TB) disease, where laboratories play a key role, is a prerequisite for any successful TB control programme. Extensive roll-out of various molecular-based modalities poses specific challenges for diagnostic laboratories as implementation of novel diagnostic assays in TB laboratory diagnosis requires effective time and resource management. Comprehensive data on hands-on time spent on specific assays and optimal workload in TB laboratories is scarce. The Time and Motion tool (T&M), which requires continuous and independent observations is generally considered to be one of the most reliable methods compared to other approaches for hands-on time recording. T&M is based on strict adherence to Standard Operation Procedures which are split into individual steps recorded by independent observers to minimise bias and to ensure objectivity and data portability between sites.
The aim of the study was to develop an objective means for recording actual time spent on running laboratory assays. This information could then be used nationally and internationally to determine the laboratory workload, make improvements and justify requirements for human resources. This multicentre prospective study was conducted in six EU reference TB laboratories within the European TB Reference Laboratory Network (ERLTB-Net).
A total of 1,060 specimens were tested using four routinely employed laboratory assays. The number of specimens per batch varied from one to 60 with a total of 64 recordings performed across six participating sites. Theoretical hands-on times per specimen (TTPS) for Xpert® MTB/RIF, genotyping, Ziehl-Neelsen and manual fluorescent microscopy were 00:33:02±00:12:32, 00:13:34±00:03:11, 00:09:54±00:00:53, and 00:06:23±00:01:36, respectively. Variations between laboratories were predominantly linked to times spent on reporting and administrative procedures. Processing specimens in batches can help save time in highly-automated (e.g. Line probe) assays (TTPS 00:14:00 vs 00:09:45 for batches comprising 7 and 31 specimens, respectively).
The T&M tool can be considered a universal and objective methodology contributing to a workload assessment in TB laboratories. Results of the study could help laboratory managers to justify their resource and personnel needs for implementation of novel, time-saving, cost-effective technologies and identify areas for improvement.

OP 26

European TB Laboratory Initiative: Algorithm for laboratory diagnosis and treatment-monitoring of pulmonary tuberculosis and drug-resistant tuberculosis using rapid molecular diagnostic technologies

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The European Tuberculosis Laboratory Initiative (ELI), with its secretariat at the WHO Regional Office for Europe, has developed a technical document to address the need in the WHO European Region for increasing timely and accurate detection of tuberculosis (TB) and multidrug-resistant TB (MDR-TB) through scaling-up the appropriate use of WHO-recommended rapid molecular diagnostic techniques. The document presents comprehensive algorithms for diagnosis and treatment-monitoring of pulmonary TB and MDR-TB using rapid molecular techniques recommended by WHO. With strong commitment of the Member States and continuous support from donors and partners, most techniques have already been introduced to the majority of countries of the Region, particularly in the high MDR-TB burden countries. However, to yield the maximum benefit of each technique, the appropriate and accurately timed sequence of different laboratory tests and correct interpretation and communication of results between laboratories and clinicians need to be ensured. For effective operation and efficient outcomes, sustainable financial and human resources need to be directed towards increasing testing capacities and optimizing sample transportation and data communication. This document aims to address these issues, taking the challenges and opportunities of the countries of the Region into account.

OP 27

Genomic epidemiology spanning decades of major Mycobacterium tuberculosis outbreak in a low-incidence setting: Lessons from sparse time-series analysis of a retrospective cohort study

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Over the last two and a half decades, Denmark has experienced the largest sustained outbreak of tuberculosis (TB) in Scandinavia ascribed to a single genotype. Based on nationwide strain-typing of every *Mycobacterium tuberculosis* culture-positive case since 1992, the '1112-15' outbreak has been documented to progress at a surprisingly high rate, despite an overall declining trend in total Danish TB cases. As of Spring 2017, the International Reference Laboratory of Mycobacteriology (IRLM) in Copenhagen has collected and identified isolates from more than a thousand cases belonging to this outbreak via routine MIRU-VNTR typing. In 2015, we began a project of whole-genome sequencing every available '1112-15' isolate identified between 1992 – 2014 (n=989), which is currently ~80% complete. Here, we present our first retrospective analysis of the 1112-15 dataset, based on whole-genome data from a sparse time-series consisting of five randomly selected isolates from each of the 23 years. Even if these data are derived from only 12% of the isolates, we have been able to extract important key information, such as mutation rate, conserved single-nucleotide polymorphisms to identify discrete transmission chains, and finally, the possible historical origins of the outbreak. In addition to helping us to put the outbreak in a global context, these data therefor also provide us with relevant information for future classification of new cases and ongoing monitoring of discrete transmission chains.

OP 28

VNTR typing is less reliable in predicting epidemiological links than expected, as indicated by Whole Genome Sequencing (WGS) analysis

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From 2004, variable number of tandem repeat (VNTR) typing has been applied routinely to all *Mycobacterium tuberculosis* complex isolates in the Netherlands. Patients with isolates sharing identical VNTR patterns are assigned to clusters under the assumption there may be an epidemiological link between them. Municipal Health Services (MHSs) contact clustered patients and investigate possible epidemiological relationship. Yearly, 19%-24% of VNTR clustered patients have confirmed epidemiological links. This low degree of confirmation may due to undetected transmission in the public domain.
WGS was applied to re-cluster a set of 561 isolates in 2016 and to compare the observations in WGS versus VNTR with the findings on epi-links by MHSs. Of these 561 isolates; 259 (46.2%) had unique VNTR patterns; 150 (26.7%) clustered with at least one other isolate from 2016 and 152 (27.1%) clustered with an isolate from before 2016. We considered isolates to be clustered by WGS if the genetic distance was equal to, or less than 12 SNPs, in line with the current literature.
Of the 259 isolates with unique VNTR patterns, six had 13 – 50 SNPs (2.3%), and 251 had >50 SNPs (96.9%) difference from the nearest clustered isolate. Two isolates with unique VNTR patterns had a <12 SNPs genetic distance, they differed by only one of the 24 VNTR loci.
Of the 150 isolates clustering on the basis of VNTR, 84 (56%) had a genetic distance of ≤12 SNPs from another strain. The remaining isolates had genetic distances ranging from 14 – 1,055 SNPs.
VNTR typing clustered 150 cases and WGS 84 cases. Information on epi-links was available from MHSs for 95 cases clustered by VNTR and 56 cases clustered by WGS. Of the 95 VNTR clustered cases, 39 (41%) had a confirmed epi-link, whereas using WGS 36/56 (64%) had a

confirmed epi-link.
In general, all 39 pairs of cases in 2016 that were epi-linked by MHSs had a relatively short genetic distance (≤ 12 SNPs), except for one pair that differed by 27 SNPs. For some isolates from asylum seekers, assigned to large VNTR clusters with no identified epi-links, we observed a remarkable short genetic distance, on average of six SNPs. These cases need further investigation.
WGS analysis is a more accurate tool than VNTR to predict cases that are epidemiologically linked. Clustering on the basis of WGS is more in line with the transmission of tuberculosis revealed by the investigations of MHSs. The introduction WGS as a routine tool will therefore target cluster investigation and reduce unnecessary labour intensive work at MHSs.

OP 29

Findings from Whole Genome Sequencing of Tuberculosis in a Geographically Large Canadian Province with a Diverse Population

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Prospective genotyping of *Mycobacterium tuberculosis* (*Mtb*) is now standard practice in many well-resourced mycobacteriology laboratories. However, advances in sequencing technologies have made it feasible to genome sequence large numbers of *Mtb* isolates, providing new insights into the transmission of tuberculosis and revealing the limitations of MIRU. A total of 2,290 clinical *Mtb* isolates collected in British Columbia (2005–2014), representing 99.3% of all culture-positive cases diagnosed in this period, were first genotyped by MIRU. The 1,009 (44%) clustered isolates (≥2 with identical MIRU), and a further 371 isolates of clinical interest were directed to whole genome sequencing (WGS) using the Illumina HiSeq and analysed using a bioinformatics pipeline designed for real-time clinical WGS of TB within Public Health England.
Here, we present initial results from this genomic survey. Using WGS over MIRU decreased our clustering rate from 44% to 29% using a 20-SNP threshold. Among non-Euro-American *Mtb* lineages – occurring largely in immigrants to Canada, MIRU clusters generally do not correspond to recent transmission; however, some transmission among household and social contacts is apparent, indicating that the often-used birthplace in a high-incidence setting as marker for reactivation disease is not necessarily true. One such cluster, comprising East-Asian lineage cases, expanded into a multi-year outbreak. Most large outbreaks (10+ cases) occur amongst Canadian-born, with WGS data suggesting a significant degree of geographic structure to transmission. Predicted antibiotic sensitivity was concordant with phenotypic data 90% of the time; predicted resistance had 67% concordance. Our next steps involve linking WGS results to case-level clinical and demographic data to enhance our understanding of the spread of TB in British Columbia and will ultimately improve TB control efforts and permit effective allocation of resources.

OP 30

WGS analysis of clusters involving immigrants to develop tailored molecular tools to discriminate between recent transmission in the host country and new importations

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Whole genome sequencing (WGS) is transforming the way in which we can analyze tuberculosis (TB) transmission. It has increased our discriminatory power to define clusters and allows us to obtain networks which inform

about the topology and direction of transmission. The analysis of transmission is challenging in populations with a high proportion of immigrants, where clusters with different composition of nationalities and combining both autochthonous and immigrants can be identified. In this context, standard molecular epidemiology based on MIRU-VNTR could face certain limitations when analyzing strains which are prevalent in the country of origin of these immigrants. Standard genotyping could fail in differentiating TB cases due to transmission of an imported strain in the host country, from other cases which are the result of new independent importations of the same strain. Our purpose was to address this question by selecting for WGS analysis a subset of clusters from a systematic molecular epidemiology program run in Almería, South-East Spain, a population with a high proportion of immigrants. We selected clusters, including cases with identical 24 locus-MIRU-VNTR patterns, enriched in immigrants from three different countries, representative of East-Europe (Romania), Maghreb (Morocco) and West Africa (Senegal). The transmission networks obtained from the WGS data and the determination of the number of SNPs between the isolates allowed us to differentiate, within each cluster, cases truly due to recent transmission (< 12 SNPs) and cases more likely representative of independent importations. Once identified the branch in the network which included the subset of patients specifically involved in recent transmission, we used the specific SNPs for that branch to design a strain-specific-PCR. This allele-specific-PCR (ASO-PCR) was prospectively applied, directly on respiratory specimens, to identify new secondary cases from the active transmission event, and to differentiate them from those corresponding to independent importations. We will apply the ASO-PCR-based strategy to identify additional representative isolates from a central collection in Morocco. The integrative WGS analysis of all the isolates will allow us to refine the complete phylogenetic picture of strains that are causing secondary cases at the host country and also circulating in the country of origin of immigrants. Based on this analysis, new tailored molecular tools will help us to optimize the tracking of these strains.

Funding: ISCIII: ERANET-LAC (TRANS-TB-TRANS REF AC16/00057), FIS (13/01207; 15/01554), cofunded by ERDF Funds from the European Commission: “A way of making Europe”. Miguel Servet grant (CP15/00075) for LPL. Ministerio de Economía y Competitividad (grant SAF2016-77346-R), ERC (638553-TB-ACCELERATE) to IC. FPU13/00913 (Ministerio de Educación y Ciencia) to ACO.

OP 31

Evolution of Extensively Drug-resistant Mycobacterium tuberculosis in Western Cape Province of South Africa: Whole Genome Sequencing of Serial Patient Isolates

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South Africa was one of the countries with the highest tuberculosis (TB) burden globally in 2015 with an incidence rate of 834 cases/100,000 population. Furthermore, the country has a very high rate (719 cases in 2015) of extensively drug-resistant TB (XDR-TB) that requires an estimated 45% of the country’s TB control budget.
It has previously been shown that XDR-TB can evolve within a patient during treatment, but the underlying mechanisms driving the evolution of XDR-TB are still not fully understood.
To investigate the genomic changes and the chronology leading to the development of XDR-TB during treatment, we performed whole genome sequencing on 200 serial *Mycobacterium tuberculosis* isolates from 42 patients of the Western Cape Province in South Africa. Isolates were selected based on routine drug susceptibility testing showing evolution from multidrug-resistance to pre-XDR-resistance to XDR-TB while on treatment. Preliminary data analysis of the genomic changes detected and their within-patient frequencies reveal several different underlying heteroresistant sub-populations and different mutation frequency patterns for almost all patients. While for some patients sub-populations appear to have resulted from a reinfection with a very similar strain, clonal microevolution appears to be the cause of the observed variation in other patients.
Preliminary data demonstrate the complex evolutionary dynamics during a TB infection and suggest that standardized, routine diagnostic procedures may fail to determine the full drug resistance profile of a patient, therefore leading to partially empiric treatment regimens with decreased effectiveness. Therefore, in the future, whole genome sequencing might be considered for diagnostic purposes to ensure a comparably fast and discriminatory method to determine the complete drug-resistance profile of each individual patient and to avoid the emergence of incurable TB.

OP 32

The Phylogeny and Taxonomy of the Genus Mycobacterium

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The phylogenetic relationships between, and definition of, mycobacterial species has been revised numerous times. Original definitions based upon phenotypic characteristics were then confirmed or challenged by genomic approaches such as 16S comparisons. Although high levels of agreement are observed between these approaches, they still suffer from many drawbacks, such as convergent evolution and low resolution. However, the increasing availability and plummeting costs of whole genome sequencing (WGS) approaches allow for its integration into taxonomic and phylogenetic studies.
WGS information derived from 41 *Mycobacterium* species and strains was used to reconstruct the phylogeny of the genus, using both ortholog concatenation and gene presence/absence. These approaches both agreed with each other, confirming the minimal effect of lateral gene transfer within this group, and with previous 16S-based approaches. However, the *M. simiae* complex was not found to be monophyletic by this robust method.
Taxonomic investigation was also undertaken on an expanded set of 134 genomes, representing 74% of currently named species. The whole genome comparison approaches Average Nucleotide Identity (ANI), Genome-to-Genome Distance (GGD) and Genomic-Signature-Delta-Difference (GS-DD) were employed to investigate the current taxonomic labels of species within the genus. This confirmed many existing species but suggested some fusions of currently separated species such as *M. conceptionense* and *M. senegalense* where no whole-genome comparisons gave weight to support their separation as different species. Also observed was the high impact of convergent evolution on the composition of helix 18 of the 16S rRNA gene, discounting this helix as a reliable taxonomic marker, as has been used in the past.
Overall, we present a variety of whole genome-based approaches that allow for investigation of both the phylogeny and taxonomy of the *Mycobacterium* genus. We suggest these should be the core toolset for defining new species and relationships, due to the lower susceptibility to convergent evolution than is associated with other historical approaches.

OP 33

Nocosomial outbreak of NTM of the Mycobacterium fortuitum complex – Detection of transmission by Whole Genome Sequencing

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Worldwide nontuberculous mycobacteria (NTM) are causing an increasing number of soft tissue infections of surgical sites, which may become infected after the wound is exposed directly or indirectly to contaminated tap water or other possible sources of infection, including implanted devices. The opportunistic pathogen *Mycobacterium fortuitum* is an environmental NTM, which belongs to the rapidly growing mycobacteria (RGM) and most commonly causes localized skin and soft tissue infections, especially common after breast surgery. Here we report on four post-operative wound infections with *M. fortuitum* complex detected in a department of surgery in Slovenia. In all four cases bacteria cultivated from wound swaps were first interpreted according to API CORYNE as *Corynebacterium jeikeum*, identified as *M.*

senegalese by MALDI-TOF and later on as *M. fortuitum* complex by sequencing of *rpoB* as well as one environmental sample from a water tap, showing the difficulties in correct species determination of NTM in clinical microbiological laboratories. Using Whole Genome Sequencing (WGS) and *de novo* assembly of reference genomes we were able to show that three of the four patient isolates and the water tap sample were highly identical, usually indicating direct transmission. This evidence strongly supports that the water supply was the source of infection and shows the advantage of WGS in tracing bacterial pathogen transmission.

OP 34

Mycobacteria in aquarium fish: poor awareness of their zoonotic potential

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The findings of our past surveys related to screening for the presence of mycobacteria in aquarium fish sold in pet-shops in Slovenia indicated a high level of ornamental fish contamination with environmental mycobacteria potentially pathogenic for humans. The predominant mycobacteria identified were *Mycobacterium fortuitum* and *M. marinum*, the latter being the most important causative agent of skin mycobacteriosis in humans. The purpose of present work was to evaluate the awareness of aquarium hobbyists and pet shop salespersons about the health risks connected to aquarium fish handling.
A total of 198 persons participated in the study. 76% of respondents were aquarium hobbyists (AH) and 24% pet shop salespersons (PSS) with mostly up to 15 years of fish handling history. There were several differences revealed between the two groups of respondents. The PSS reported getting education and reading the literature in a higher proportion. Additionally, we found out that AH often perceive PSS as a relevant source of information. While the majority (74%) of PSS agreed that aquarium fish can get sick, only 25% of AH shared the same opinion. About one third (36%) of respondents were positive that some fish diseases can be transmitted to humans. Similarly, 36% confirmed that fish may contract tuberculosis (TB). However, only 24% of respondents agreed that fish TB is a zoonotic disease, where PSS are obviously more aware of fish TB and its zoonotic potential. Furthermore, 11% claimed that they recognize clinical signs of fish TB (among them, PSS showed statistically significant predominance) while only 3% have ever heard of fish tank granuloma. Considering the general belief expressed by 75% of respondents that aquarium water may pose a risk to human health, their aquarium handling practices are surprising as the majority (75%) never use waterproof gloves when cleaning the aquarium or handling fish and 67% never disinfect the aquarium after changing the water. Almost two thirds of respondents always clean the aquarium after changing the water though. Waste water is mainly poured down the sink (83%) while the others mostly use it for watering the flowers or garden. Dead fish are frequently flushed down the toilet by AH (52%) or frozen and delivered to an authorized service by PSS (46%).
In conclusion, more effort should be put into increasing the awareness about the role of mycobacteria in infections associated with exposure to aquarium fish, especially among AH.

OP 35

Exploring the clinical relevance of NTMS causing pulmonary and extra pulmonary diseases in Saudi Arabia: Findings of the first national surveillance study. Exploring the clinical relevance of NTM mycobacteria causing pulmonary and extra pulmonary diseases in Saudi Arabia

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Non-tuberculous Mycobacteria (NTM) causing diseases are increasing worldwide without exception to any geographical regions. Saudi Arabia also faces the same global trend of increasing prevalence of NTM diseases. However, there are limited data on the real disease and species spectrum of NTM's. To understand the consequences of clinical NTM diversity, the first nationwide surveillance study has been conducted during April 2014- November 2015. A non-repetitive culture confirmed, 527 NTM isolates from different provinces of the country

were collected with all the clinical data. Primary identification was carried out by using line probe assays followed by sequencing of *16S rRNA*, *16S-23S ITS* region, *rpoB* and *hsp65* genes.
The study population was dominated by Saudi nationals (75.2%) with a male (65.3%) gender predominance and a mean of age 46.1 years. Geographical distribution of cases showed the Western and Central provinces of the country have the highest rate of NTM diseases. A total of 396 (75.1%) were pulmonary and 131(24.9%) were extra-pulmonary cases. Among extra-pulmonary cases, lymphnode (33%) remained as the most common site of infection mainly followed by skin (24%), gastrointestinal sites (14%) and 10% cases of mycobacteremia. Strictly following the American Thoracic Society guidelines, 174 (43.9%) pulmonary cases were confirmed as clinically relevant disease. Analysis on predisposing conditions showed diabetes mellitus (15.2%) and previous history of pulmonary tuberculosis (12.3%) as the most common factors. HIV prevalence among the study population was only 2.7%.
Species spectrum of NTM's in the study revealed 34 established species and 1.5% isolates remained with undefined identity. *M.simiae* (22.6%) was the most predominant species mainly followed by *M.abscessus* (17.8%), *M.fortuitum*(17.6%), *M.gordonae* (7.6%) and *M.intracellulare* (6.5%). For the first time in the country clinically confirmed pulmonary and extra-pulmonary diseases caused by 18 rare species were observed. Among them, multiple cases caused by *M.monascence*, *M.cosmeticum, M.iranicum, M.kubicae, M.duvalli, M.terrae and M.triplex* were identified. Surprisingly, an unexpected emergence of *M.riyadhense* (5.3%) as a common pathogen was found during the study period dominating among Saudi nationals. In conclusion, the findings showed an increasing of various NTM species as established pathogens in all the country. The current scenario of NTM's as neglected pathogens must be changed immediately to better manage clinical consequences of the diseases.

OP 36

Nontuberculous mycobacteria in a tertiray hospital in Portugal and their clinical significance

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Background: Nontuberculous mycobacteria (NTM) and their clinical relevance are poorly understood. Evidence suggests a growing incidence, but their true distribution remains largely unknown. To further understand the clinical relevance of NTM, the demographic and clinical characteristics of patients who had positive cultures for NTM at a tertiary hospital in Portugal were reviewed.
Material/methods: Retrospective analysis of patients assessed at the Infectious Diseases Department of the São João Hospital Center (SJHC), from January 2007 through December 2014, from whom at least one biological sample was tested culture-positive for NTM.
Results: A total of 74 patients with at least one positive culture for NTM were identified. Out of the 74 patients, 33 (44.6%) were current or ex-smokers, 13 (17.6 %) had a diagnosis of chronic obstructive pulmonary disease (COPD) and 7 (9.5%) had bronchiectasis. Forty-nine of them (66.2%) were infected by the human immunodeficiency virus (HIV), four (5.4%) had cancer, and seven (9.5%) were under immunosuppressive medication. Eleven of them (14.9%) had a history of Tuberculosis (TB). A total of 13 patients (17.6%) fulfilled the current American Thoracic Society/Infectious Diseases Society of America (ATS/IDSA) criteria for pulmonary NTM disease and treatment was also initiated in 12 other patients (16.2%) considered to have clinical evidence of NTM disease, all of which were immunocompromised (10 had HIV infection and 2 were under immunosuppressive medication). In total, 25 patients were treated for NTM disease (33.8%). The majority of the isolates (47%, n=35) were retrieved from respiratory samples and the most common species isolated were *M. avium complex* (MAC) (35.1%, n=26), followed by *M. gordonae* (28.4%). In total 9 different species were isolated. MAC was more frequently associated with disease, responsible for 56% of the patients treated. Patients were treated with drugs adjusted for the species isolated, and cure was achieved in 13 patients (52%).
Conclusions: Mycobacteria form a very heterogeneous group and some species are clearly more clinically relevant than others. Also, through aging of the population, new forms of immunosuppression and the advent of HAART (highly active antiretroviral therapy) for HIV patients, their distribution seems to be shifting. The present study highlights the importance of understanding the epidemiology of NMT to better comprehend their clinical impact.

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ABSTRACTS OF POSTER PRESENTATIONS (P)

NGS diagnostics and transmission analysis

P 21

Changes in nitric oxide status in patients with or without multi-drug resistant tuberculosis

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Aims and objectives: There is a paucity of published data on the effect of TB on nitric oxide (NO) synthesis and metabolism in newly diagnosed and relapsed patients with or without multi-drug resistant tuberculosis (MDR-TB).
Methods: The pattern of NO response in 140 patients with pulmonary TB, including 74 with MDR-TB (1st group) and 66 without MDR-TB (2nd group) has been studied and compared to the NO status of 30 healthy donors (3rd group). Patients comprised those with newly diagnosed TB (NDPTB) (Subgroups 1B,2B) and recurrent or relapsed TB (Subgroup 1A, 2A). The NO status was assessed by measuring inducible NO synthase (iNOS), nitrites and nitrates levels. This was measured prior to treatment initiation.
Results: Increased levels of NO indices were found in patients with TB when compared (1st group (iNOS-231.6±6.65 pmole/min/mgB, nitrites - 5.626±0.15 µmol/L and nitrates - 62.89±1.42 µmol/L) Subgroups 1A (iNOS-208.40±8.26 pmole/min/mgB, nitrites-5.027±0.17 µmol/L and nitrates - 59.29±1.79 µmol/L) and Subgroups 1B (iNOS - 260.4±8.56 pmole/min/mgB, nitrites - 6.371±0.19 µmol/L and nitrates - 67.36±2.03 µmol/L)) and 2nd group (iNOS - 286.3±5.92 pmole/min/mgB, nitrites-6.747±0.17 µmol/L and nitrates-72.02±1.43 µmol/L) Subgroups 2A (iNOS - 260.9±14.12 pmole/min/mgB, nitrites-5.686±0.20 µmol/L and nitrates - 66.26±1.89 µmol/L) and Subgroups 2B (iNOS - 293.7±6.13 pmole/min/mgB, nitrites - 7.059±0.19 µmol/L and nitrates - 73.72±1.71 µmol/L))) to healthy controls (iNOS - 81.03±2.36 pmole/min/mgB, nitrites - 3.83±0.093 µmol/L and nitrates - 37.98±1.30 µmol/L).
Conclusion: In patients with pulmonary TB, significantly higher levels of NO activity were observed as compared with the levels in healthy individuals. In patients with recurrent TB and MDR-TB, significantly lower levels of NO indicators were observed by comparison with patients with newly diagnosed pulmonary TB. Therefore, the levels of nitrites and nitrates aswell as iNOS activity may serve as additional diagnostic criteria to differentiate MDR-TB from non-resistant TB in patients with relapsed and newly diagnosed TB.

P 40

Systematic evidence for the performance of Whole Genome Sequencing for detection of M. tuberculosis drug resistance

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Whole Genome Sequencing (WGS) offers new opportunities in the clinical management of drug resistant tuberculosis (TB) cases, providing information with unprecedented accuracy when compared to current routine methods but systematic data with regards to the role of WGS of *M.tuberculosis* (MTB) for the prediction of drug resistance and drug susceptibility are lacking. We conducted a systematic review to determine the diagnostic accuracy of MTB WGS for the detection of resistance to first and second line anti-TB drugs. Data was extracted from 20 publications (out of 2,492 originally identified) and sensitivity and specificity of WGS vs phenotypic Drug Susceptibility Testing (DST) methods were determined.
Polymorphisms in 53 genes were tested for associations with resistance to a total of 22 drugs. Pooled sensitivity and specificity values for detection of resistance to rifampicin and isoniazid were 0.98 (95% CI, 0.93 to 0.98) and 0.98 (95% CI, 0.98 to 1.00); 0.97 (95% CI, 0.94 to 0.99) and 0.93 (95% CI 0.91 to 0.96), respectively. Due to

high heterogeneity in studies' design, lack of knowledge of resistance mechanisms and clarity on exclusion of phylogenetic markers, there was a significant variation in analytical performance of WGS for the remaining first-line, reserved drugs and new drugs.
Overall WGS could be considered a promising alternative to existing methods for rifampicin and isoniazid resistance detection in TB cultures pending standardization of analytical pipelines' software parameters, databases and other resources specifying the role of specific genes and mutations in the development of drug resistance.
The high variations in WGS analytical performance for specific drugs highlight the importance of the development and standardization of post-processing procedures and algorithms, including extensive validation of resources containing information on high- and low-level resistance conferring mutations vs phylogenetic polymorphisms collected through large population-based studies conducted different geographical areas and epidemiological settings. To ensure clinical relevance of WGS, future studies should include information on clinical outcomes.

P 42

Direct MIRU-VNTR genotyping of Mycobacterium tuberculosis from Xpert MTB/RIF remnants

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Genotyping of *Mycobacterium tuberculosis* (MTB) isolates has markedly improved our knowledge of the transmission dynamics of this pathogen. MIRU-VNTR is currently considered the reference molecular tool for MTB fingerprinting. However, the dependence of this technique on cultured isolates means that we lack molecular epidemiology data from many settings where culture facilities have not been implemented yet. Efforts have been made to adapt the MIRU-VNTR procedure to direct analysis of clinical specimens, although implementation of these efforts has not proven successful. The large-scale roll-out of Xpert® MTB/RIF (Xpert) technology, which is now available in almost every TB-endemic country, including many where MTB is not routinely cultured, allows us to explore whether MTB molecular analysis could be performed directly from the remnants of the Xpert cartridge. We initially succeeded in using Xpert remnants from Equatorial Guinea delivered to Spain when used as templates for Allele Specific Oligonucleotide-PCRs. However, when MIRU-VNTR was performed on the same remnants, poor results were obtained, leading to incomplete MIRU-types. We interpreted that the prolonged storage at room temperature before being delivered to Spain could affect to the quality of the DNA. Therefore, we evaluated "*onsite*" the performance of this strategy in Mozambique, a setting with a high TB incidence rate and suitable laboratory capacities. Our aim was to use the remnants from the sample chamber of 24 Xpert-positive assays to obtain MIRU-VNTR data for the 15-locus or the more discriminatory 24-locus format. The study included samples with different bacterial load and different periods of time since Xpert was performed. Our results showed that MIRU-VNTR analysis was possible from Xpert remnants from positive specimens with high bacterial load, and when the delay between Xpert test and the fingerprinting analysis was shorter than 1 week. We are currently evaluating whether the MIRU-VNTR performance can be improved by using the remnants from the cartridge internal chambers which harbour the material subjected to sonication during the Xpert assay. Given the broad roll out of Xpert availability and its simple processing needs, our findings suggest that MIRU-VNTR–based fingerprinting from remnants of Xpert could play a major role in extending MTB molecular epidemiology studies to settings where information on the transmission dynamics of this pathogen is lacking.
Funding: ISCIII: ERANET-LAC (TRANS-TB-TRANS REF AC16/00057), FIS (15/01554),

cofunded by ERDF Funds from the European Commission: "A way of making Europe". IiSGM (I and II-COOP-INT 2015). Miguel Servet research grant (CP15/00075) for LPL, Erasmus Mundus Joint Doctorate Program of the European Union (Transglobal Health) for ALGB.

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Detection of low-level resistance in tuberculosis bacteria using next-generation sequencing approaches

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Tuberculosis (TB) is still one of the leading infectious diseases globally. Increasing numbers of antibiotic resistant cases threatens worldwide TB control and remains a major public health problem in many countries. The early diagnosis is indispensable to prevent treatment failure and spread of resistant bacteria. The valid phenomenon of small numbers of drug resistant TB in a bacterial population, also known as heteroresistance, tends to pose problems for current standard methods. As a consequence untreated low-level drug resistant subpopulation in clinical TB samples could lead to treatment failure.
Conventional drug susceptibility testing (DST) using the 1% proportion method aims to determine if 1% or more of the bacterial population in clinical specimens is drug resistant, but it is not quantitative and time-consuming as it need several weeks to complete. Faster molecular diagnostic test such as the line probe assays or Sanger-sequencing are not able to reach a similar biological relevant sensitivity to detect heteroresistance. Although whole genome sequencing using Next-generation sequencing (NGS) methods has the potential for simultaneous detection of wild-type and mutated alleles, recent publications reported a threshold of 30% resistant alleles to be detectable.
To address these challenge we implemented a variant detector which not only detects but also statistically rates variants in resistance mediating genes.
We generated 6 mixtures of susceptible and two different rifampicin mono-resistant strains, with 1%, 5% and 10% resistant bacteria, respectively. The samples where sequenced using 500 times coverage on an Illumina MiSeq system.
We simulated different coverages to get the theoretical detectable threshold of 2% resistant bacteria with at least 400 reads coverage and mean base quality. However, our method showed 100% sensitivity and 100% specificity to detect the low-level *rpoB* mutations in all proportions tested, even for 1% resistant bacteria.
This method is beneficial to make NGS-based diagnostics become more reliable in future.

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The evolution of Mycobacterium tuberculosis lineages analyzed by a combination of wgMLST and wgSNP

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Introduction: Tuberculosis (TB) is one of the world's deadliest diseases with one third of the world's population being infected. Yearly, over 10 million people become sick worldwide, resulting in over 1.5 million TB-related deaths worldwide. A globally accessible analysis method for WGS data to identify outbreaks, to define the source of infection and to disclose its routes of transmission and dissemination in the environment is key, and data exchange based on a common nomenclature is necessary to optimize global TB surveillance.
Methods: In this study, two high resolution WGS-based typing methods were performed using the BioNumerics software. Calculation-intensive data processing steps were executed on the BioNumerics® cloud-based calculation environment.
First, whole genome multi-locus sequence typing (wgMLST) was applied based on a pangenome MLST scheme which is a combination of the core genome MLST definition as published by *Kohl et al.*(1) and additional allelic diversity identified in a set of 46 publicly available reference sequences, among which *M. bovis*, *M. africanum*, *M. canettii* and prototuberculosis strains. Two independent allele calling approaches were applied, an assembly-free and a BLAST-based allele calling algorithm, to determine locus presence and detect allelic variants.
Subsequently, a cluster defined by wgMLST can be further characterized by whole genome single-nucleotide polymorphism analysis (wgSNP). The wgSNP pipeline, tuned to reduce false positives while maximizing resolution, detects SNP variants by mapping the WGS reads to a reference sequence to further maximize resolution.
Results: wgMLST clearly proved suitable for the rapid analysis of large datasets, making it a useful technique for outbreak surveillance and providing additional resolution to cgMLST analysis. In addition, wgSNP provides the utmost resolution increasing the confidence in detected clusters.
BioNumerics offers a powerful platform where both wgMLST and wgSNP analysis can be

performed in a robust, reproducible manner and at high-throughput rates. Moreover, the publicly available nomenclature allows for a portable and high resolution exchange of data and results.
Conclusion: The combination of two complementary approaches, wgMLST and wgSNP, on a virtually unlimited number of samples, managed by a single software platform that also stores traditional data as well as metadata, permits detection of subtype- or outbreak-specific markers, enabling powerful classification and outbreak detection.
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Tuberculosis in indigenous population in Puerto Nariño, Amazonas, Colombia

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Introduction: In 2015, Colombia reported 12 918 TB cases for an incidence of 24.2 cases per 100,000 inhabitants. The department that showed the highest incidence was Amazonas (72.1 / 100 000). Indigenous peoples have been declared by different health agencies as a population with a great risk of tuberculosis transmission. In Colombia, 5.3% of cases reported in 2015 (689 cases) were in indigenous population, from which 11.9% were in people younger than 15 years old. The main goal of the study was to determine the situation of active tuberculosis in Puerto Nariño municipality (Amazonas department), Colombia.
Methods: Cross-sectional descriptive study. An active search for respiratory symptoms of any duration was carried out through medical examination to those who signed informed consent. Serial spontaneous or induced sputum samples were taken to perform microbiological diagnosis: Ziehl–Neelsen staining, cultures (LJ and BACTEC™ MGIT™) and phenotypic/ genotypic drug susceptibility testing.
Results: 5837 individuals were enrolled. A total of 944 individuals were identified with respiratory symptoms (16.2%) and 76 patients were diagnosed with TB by culture and/or ZN stain. A single clinical isolate (1.3%) was confirmed as MDR-TB and the others were sensitive to first-line drugs. By Spoligotyping, all isolates were confirmed as *M. tuberculosis* species corresponding to Euro-American lineage 4, with a predominance of the LAM sublineage 54.3%. The subfamilies found were: LAM6 36/70 (51%), LAM9 2/70 (3%), and T2 31/70 (44%).
Conclusions: Incidence rate (954.2/100000) was 37.3 times higher than that was reported at the national level (25.6/100000). It was determined that 41.3% of positive cases are in children under 15 years old which indicates the importance of diagnosis in this population group. A case of MDR-TB was found within the study. Prevalent Tb subfamilies were LAM6 and T2.

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Transmission analysis of a multidrug-resistant Mycobacterium tuberculosis outbreak in Bulgaria

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Context: More than 130 MDR strains of SIT41 (TUR) genotype were identified, 2008-2016. Molecular typing confirmed strong association between MDR and SIT41 (TUR) MTB sublineage. The cases with SIT41 strain genotype is>40%, while the drug sensitive strains of SIT41 genotype represented by only 2%. SIT41 genotype is considered as a marker for MDR tuberculosis in Bulgaria.
Aim: Our study intended to apply WGS analysis to reconstruct the transmission dynamics of an MDR outbreak at higher resolution than 24 MIRU-VNTR analyses.
Methods: We sequenced the complete genomes of 35 *M. tuberculosis* isolates. The sequencing analysis of additional 45 isolates is ongoing.
Results: The results of 24 locus MIRU-VNTR analysis identified three clusters including 17, 26 and 67 strains. Five other clusters

include between 3 and 9 strains. The largest dominant cluster MDR MIRU-VNTR profile 244124132134425113333b32 was identified all over the country. This genotype is not common in other countries, suggesting local evolution. The highest concentration of the strains was in the North-West part of Bulgaria.

Conclusions: WGS is ongoing. Preliminary results revealed several concomitant transmission events leading to independent micro-epidemics historically originating from a common ancestor genotype.

***Acknowledgements*:** This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 660150.

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International validation of Whole Genome Sequencing (WGS) of Mycobacterium tuberculosis (MTB) isolates

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WGS provides the highest resolution for MTB epidemiological typing and the detection of antibiotic resistance associated mutations. The analysis and sharing of the comprehensive complex WGS data results in a need for standardization. Bias may result from differences in sequencing technique, mapping and SNP calling algorithms, or the in/exclusion of specific genomic regions. Such variation may alter the exact number of SNPs recorded between pairs of strains by different laboratories.

In a collaborative project, strains from an international VNTR cluster shared by Denmark and the Netherlands were exchanged and subjected to WGS analysis at both laboratories. Comparable phylogenetic trees were constructed at both sites but many fewer SNPs were reported using the Danish pipeline. This was mainly caused by the exclusion of repetitive regions in the MTB genome.

Thirteen laboratories from different European countries that participated in the ERLTB-Net WGS 2016 proficiency study were asked to share their raw sequence data on the panel of five DNA samples, of which two were duplicates, to identify the effect of sequencing technology, i.e. MiSeq, HiSeq, NextSeq, Ion Torrent, and MiniSeq, on WGS results. The raw sequence data were analyzed at the RIVM using two WGS analysis methods: 1) analyzing all genetic regions; and 2) a more stringent method excluding repetitive regions annotated as PE/PPE, PGRS, pks, esx, repeat, polyketide, pks, and transposase.

There was much lower genetic distance between the duplicates compared to the other samples, however a genetic distance between 16 and 47 SNPs remained if all genetic regions were analyzed. When analyzing the duplicates according to the stringent pipeline, a maximum of six SNPs difference between the duplicates was observed, the two NextSeq and one HiSeq sequence data sets revealed zero SNPs differences.

Comparing the same samples between all laboratories, a genetic distance up to 116 SNPs was observed when all genetic regions were included in the analysis, this declined to a maximum of 26 SNPs when the stringent pipeline was applied.

The sequencing technique influenced the results less than the analysis pipeline. Agreeing on a standard list of genetic regions to exclude from the raw data analysis would aid international studies exploring MTB WGS.

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Laboratory implementation of Next Generation Sequencing for medium and high troughput whole genome analysis of clinical M. tuberculosis complex strains

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Tuberculosis (TB) remains the most deadly bacterial infectious disease worldwide. Treatment and control of TB is threatened by increasing numbers of multi-drug-resistant (MDR) or nearly untreatable extensively drug-resistant (XDR) strains. Therefore, new concepts are urgently needed to improve patient care through faster and comprehensive diagnostics, and to improve our understanding of the factors driving the TB epidemics, especially the spread of drug resistant outbreak strains.

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). Simultaneously, it allows for highest-resolution genotyping for outbreak analysis or longitudinal genome based molecular epidemiological studies. The application of WGS as a standard tool for diagnostics and genotyping has become realistic due to the decreasing costs per base sequenced and the development of so-called benchtop NGS systems. However, integration into a normal laboratory workflow is still challenging for non-specialized laboratories.

Here, we describe the implementation of NGS sequencing technology in low, medium, and high throughput workflows using three different Illumina sequencing machines: the MiniSeq, MiSeq, and NextSeq instruments. Working with customized preparation protocols for different sample materials (cultures, lysates, frozen stocks) allows to employ NGS analysis for different study designs ranging from retrospective large-scale case collections to ad hoc requests for NGS based characterizations of clinical samples. All workflows include rigorous quality control steps including the generated sequencing data. Importantly, the protocols are easily adaptable to other bacterial species, and were already successfully used for different mycobacterial species as well as other pathogens of interest. Of especial interest to small to medium settings, we can reach a turnaround time of less than one week for the characterization of clinical samples. This can be coupled with the extremely quick and fully automated web-based NGS interpretation pipeline PhyResSE (https://bioinf.fz-borstel.de/mchips/phyresse/), directly generating genotyping and resistance reports from uploaded sequence data.

In conclusion, we successfully implemented efficient and flexible workflows for NGS based genomics using three different Illumina sequencers together with customized protocols. This allows for optimized application of NGS for different diagnostic and/or research questions.

P 4

Delivery of Mycobacterium tuberculosis lipids using chitosan nanoparticles induce potent cytokine and antibody response through activation of gamma delta T-cells in mice

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Activation of cell mediated and humoral immune responses to *Mycobacterium tuberculosis* (Mtb) are critical for protection. Herein, we show that mice immunized with Mtb lipid bound chitosan nanoparticles(NPs) induce secretion of prominent Th1 and Th2 cytokines in lymph node and spleen cells, and also induced significantly higher levels of IgG, IgG1, IgG2 and IgM in comparison to control mice. Furthermore, significantly enhanced γδ-T cell activation was observed in lymph node cells isolated from mice immunized with Mtb lipid coated chitosan-NPs as compared to mice immunized with chitosan-NPs alone or Mtb lipid liposomes. In comparison to $CD8^+$ cells, significantly higher $CD4^+$ cells were present in both the lymph node and spleen cells isolated from mice immunized with Mtb lipid coated chitosan NP. In conclusion, this study represents a promising new strategy for efficient delivery of Mtb lipids using chitosan NPs to trigger enhanced cell mediated and antibody response against Mtb lipids. Also this delivery system can be promising vaccine candidate aganist TB.

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Mutations, level of resistance and treatment outcomes of isoniazid-resistant tuberculosis in Brazil

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Background: Mutations in *kat*G and *inh*A, resistance levels and treatment outcomes of isoniazid (INH)-resistant TB patients from the state of São Paulo, Brazil, were described.
Methods: We analysed all INH-resistant TB cases with *M. tuberculosis* isolates identified at Instituto Adolfo Lutz from October 2008 to March 2009 (1 isolate/patient was included). INH at 1µg/mL (low-level), 3µg/mL (intermediate) and 10µg/mL (high-level resistance) was tested by MGIT960. *kat*G 315 codon and *inh*A promoter region were sequenced. Patients' data were collected from TB-WEB, an information system in which TB cases from São Paulo are notified. From a total of 97 isolates, 54(55.7%) were MDR, 31(32%) INH-monoresistant and 12(12.3%) polyresistant.
Results: 92/97 isolates had INH resistance level valid results. Of the 52 MDR isolates, 30(57.7%) showed low-level, 10(19.2%) intermediate and 12(23.1%) high-level resistance. Of the 28 monoresistant isolates, 17(60.7%) showed low-level, 7(25%) intermediate and 4(14.3%) high-level resistance. Of the 12 polyresistant isolates, 2(16.7%) were low-level, 7(58.3%) intermediate and 3(25%) high-level resistant. Monoresistant and MDR isolates showed low-level resistance when compared with intermediate or high-level resistance ($p<0.001$). 50/97 (51.5%) isolates had mutations in *kat*G only and 5(5.2%) in *kat*G and *inh*A, while 18(18.6%) had mutation in *inh*A -15 only (all had the G-A substitution). No mutation was found in 24(24.8%) isolates. The S315T substitution was detected in 50/55(90.9%) isolates with mutations in *kat*G. Resistance levels were obtained for 49/50 isolates with mutations only in *kat*G: 23(46.9%) showed low-level, 22(44.9%) intermediate and 4(8.2%) high-level resistance. Of the 5 isolates with mutations in *kat*G and *inh*A, 4 showed high-level and 1 intermediate resistance. High-level resistance was associated with mutations in *kat*G and *inh*A simultaneously when compared with *kat*G alone ($p=0.001$). High-level resistance was found in 7/20(35%) isolates with no mutation and in 4/18(22.2%) isolates with the *inh*A -15 G-A substitution. 50/97(51.5%) patients had previous history of TB. 35(36.1%) patients were cured, while 58(59.8%) had unfavourable outcomes and 4(4.1%) were lacking information. Among the patients cured, 6(17.1%) relapsed, 5 of which had the S315T substitution.
Conclusion: The majority of isolates showed low-level resistance to INH. High-level resistance was detected in isolates expected to be genotypically low-level resistant.
Keywords: *Mycobacterium tuberculosis*; drug resistance; isoniazid; treatment outcome

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Expression of mycolic acids in pulmonary and extrapulmonary clinical isolates of M. tuberculosis under surface stress

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Tuberculous lymphadenopathy is a diagnostic and therapeutic challenge as it mimics other pathologic processes. Additionally, it is still not clear why *M. tuberculosis* causes pulmonary tuberculosis (TB) in some individuals and extra pulmonary TB in others.
The present study analyzed the mycolic acid expression in clinical isolates of *M. tuberculosis* from pulmonary TB (PTB) and lymph node TB (LNTB) to obtain an insight into these differential disease manifestations.
A total of 247*Mycobacterium tuberculosis* PTB and 13 LNTB clinical isolates were collected. All the isolates were confirmed to be *M. tuberculosis* by biochemical tests and PCR restriction analysis. Drug susceptibility testing to isoniazid, rifampicin, streptomycin and ethambutol was performed by proportion method. *M. tuberculosis* H37Rv and clinical isolates were exposed to surface stress (0.05% SDS containing broth). The mycolic acid content was analyzed by Thin layer chromatography (TLC). Expression of mycolic acid synthesis genes was tested by Real Time PCR using the housekeeping gene *sigA.*
Of the twenty drug sensitive *M. tuberculosis* clinical isolates including 10 PTB and 10 LNTB isolates selected for the study, the expression of α-mycolic acid during exposure to SDS was high in six isolates of PTB and seven isolates of LNTB. Methoxy mycolic acid showed an increased expression in five PTB isolates and seven LNTB isolates, whereas, ketomycolic acid showed increased expression in only three PTB isolates as against eight LNTB isolates. *fas* gene was upregulated in three PTB isolates and six LNTB *M. tuberculosis* isolates. *inhA* and *pks13* showed similar expression in PTB and LNTB isolates.
Thus, during surface stress, all three mycolic acid components were expressed in more number of LNTB isolates than PTB isolates. In correlation with this, *fas* was upregulated more in LNTB isolates than PTB *in-vitro*. LNTB isolates may be programmed to respond to the harsh environment arising due to surface stress better than PTB isolates.

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Mutations in Rv3805c (aftB): A probable cause of high-level ethambutol resistance in clinical isolates of Mycobacterium tuberculosis

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Introduction: Resistance against ethambutol (EMB), a first line anti-TB drug, is accounted for only in 40-60% cases where mutations in *embB* codons 306, 406 and 497 are most common. Reports regarding the presence of these mutations in EMB sensitive clinical isolates, hint towards exploring genes apart from the *embCAB* operon. Recent studies have found association of *ubiA* mutations with EMB resistance in some regions of the world. The gene *aftB,* present upstream to *ubiA,* encodes for arabinofuranosyl transferase similar to embCAB and is involved in arabinogalactan biosynthesis pathway. The present study explores polymorphisms in *aftB* for their association with EMB resistance in clinical isolates of *Mycobacterium tuberculosis.*
Method: Well-characterized clinical isolates of *Mycobacterium tuberculosis* (n=360) were subjected to drug susceptibility testing (DST) by 1% proportion method. Of these, 26 EMB resistant and 24 EMB sensitive isolates were screened for polymorphisms in *aftB*, *ubiA*, *embB*, *embC* and upstream *embA* region and further correlated with the minimum inhibitory concentration (MIC).
Result: Non synonymous polymorphisms in *aftB* were found in 7/26 (26.92%) EMB resistant strains but none of the EMB sensitive isolates. The association of the polymorphism with EMB resistance was found to be statistically significant (p=0.0101, Fischer exact test). Interestingly, the presence of this mutation was observed in high-level EMB resistance (MIC>/= 16ug/ml). However our study did not find any *ubiA* mutations associated with resistance.
Conclusion: This preliminary data proposes *aftB* as a candidate gene for studying polymorphisms leading to EMB resistance. It is also projected that, accumulation of mutations at *embB*, *embA* upstream region and *aftB* may lead to a phenotype with high-level of EMB resistance.

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HIV infection trend in TB patients in Libya

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Introduction: Globally, of the 10.4 million new incident Tuberculosis (TB) cases estimated in 2015, TB patients living with HIV accounted for 1.2 million (11%) of all new TB cases.
The relationship between HIV and TB disease is well known. HIV epidemic continues to stimulate the global TB epidemic and vice versa.
Libya is a middle income country in the Maghreb region of North Africa with over 6 million inhabitants with the majority live in the cities of coastal region.
A national Seroprevalence study, which was done 12 years ago, has revealed an HIV prevalence of 0.13% in the general population.
We aimed in this study to describe a nine years period trend of HIV incident infections among patients newly diagnosed with Tuberculosis disease in Libya during the period from 2007 to 2015.
Method: This retrospective study used aggregated data from the registries and annual reports collected by the directorate of tuberculosis and Leprosy control programme (TBP). All patients diagnosed with tuberculosis during the study period from 1 January 2007 to 31 December 2015 were included.
Data entered and analyzed by the author using excel programme
Results: HIV/TB coinfection trend:
The rate of HIV coinfection in TB diagnosed individuals between the years 2007 and 2015 is shown in figure 2. HIV/TB coinfection rate was 8.6% in 2007 and increased to 9.9% in 2008 but decreased again in year 2009 (7.1%). HIV infection has increased again to 8.9% in year 2010 and from then rates have continued to fall to finally reach 2.2% in 2015.
TB disease incidence trend:
Nationally, 2119 individuals were diagnosed with TB in 2007. Numbers have mildly decreased the next year to 2010 patients. Levels have fluctuated slightly in the next two years. Numbers then reduced steadily to reach 950 cases in year 2015.
Conclusion: A downward trend in HIV infection rates in TB cases has been noticed during the studied 9 years period.
In 2010, the National HIV Programme has introduced and promoted for HIV testing and counseling (HTC) and hotline programme for HIV education and information.
Raising awareness campaigns were also launched since then aiming at improving early diagnosis rates and retention to ART treatment. Hence the better chances of control of opportunistic infections like TB among PLHIV in the country.
The total numbers of patients diagnosed with TB disease in Libya declined dramatically in the study period. However, this reduction could be due to a real reduction in cases and infections or due to disturbed case notification system and affected accessibility to health services after 2011 revolution.
New assessment of TB case notification system is highly recommended.

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Antimicrobial Susceptibility Testing of Rapidly Growing Mycobacteria in a Clinical Mycobacteriology Laboratory

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Rapidly Growing Mycobacteria (RGM), a group of Non-tuberculous Mycobacteria (NTM), are emerging as potential human pathogens that are known to cause numerous infections in healthy and immunocompromised subjects. Isolates from patients of suspected tuberculosis (n=1079) were collected from North Delhi region. The isolates were identified by conventional methods and by PCR Restriction Analysis (PRA) with the restriction enzymes NruI and BamHI. Further speciation of NTM was done by PRA using the enzymes Sau96I and CfoI. Antimicrobial susceptibility testing (AST) was carried out for the clinically relevant RGM by Microplate Alamar Blue Assay against streptomycin, rifampicin, ethambutol, isoniazid, amikacin, cefoxitin, levofloxacin, sulfamethoxazole, clarithromycin, ciprofloxacin, tetracycline, clofazimine, tigecycline, doxycycline, linezolid and imipenem.
Out of 1079 isolates, NTM were identified in 299/1079 (27.71%) cases. Of these, 96/299 NTM that were repeatedly isolated from the same patients were taken up for further study. RGM were identified in 45/96 (46.87%) of the NTM isolated. Of these, 12/45 (26.66%) isolates were *Mycobacterium abscessus*, 31/45

(68.88%) were *Mycobacterium fortuitum* and 2/45 (4.44%) were identified as *Mycobacterium mucogenicum*. Finally, clinical relevance was attributed to 7 isolates of *M. abscessus* obtained from 4 patients based on guidelines for clinical relevance of NTM provided by the American Thoracic Society (ATS). Similarly, one isolate of *M. fortuitum* from a single patient and 2 isolates of *M. mucogenicum* from the same patient were found to be clinically relevant. All the isolates were susceptible to linezolid. Resistance to amikacin and clarithromycin was observed in (1/6) (16.66%) isolate each. Resistance to clofazamine was seen in 2/6 (33.33%) isolates. Maximum resistance was observed for doxycycline, sulphamethoxozole, tetracycline and fluoroquinolones.
Though clinical relevance was attributed to only 10 RGM isolated from 6 patients, the study highlights the importance of identifying this group of organisms. Resistance to mostly available drugs make RGM, especially *M. abscessus*, a difficult-to-treat entity.

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Retrospective Evaluation of 11 Patients with Bone and Joint Tuberculosis in Mersin, Turkey

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Objective: Tuberculosis (TB), caused by Mycobacterium tuberculosis complex (MTC), is a granulomatous infection and can be localizated in pulmonary and extrapulmonary sites. Of the all TB cases, 1-3% and of the extrapulmonary TB cases, 10-11% are located in bone and joint tissues. In this study, it was aimed to retrospectively evaluate the cases of MTC isolated, were sent with suspected bone and joint TB to the Medical Microbiology Laboratory of Mersin University, Faculty of Medicine.
Materials and Methods: Between January 2005 and December 2016, 220 patients samples with suspected bone and joint TB, were examined retrospectively. Patients with EZN and / or culture positivity were included in the study group. Additionally, the patients demographic characteristics, pathological and radiological findings, microbiologic and drug sensitivity tests results were evaluated.
Results: The MTC positivty was detected by EZN and/or culture in 11 of 220 cases with suspected bone-joint TB. The average age of patients was 27.8 (min-max: 2-64). In one case, only the EZN positivity was detected, and MTC was detected by MGIT/LJ cultures in 10 cases. Of the culture positive 10 samples, ARB positivity was detected in 6 samples. Additionally, of the culture positive isolates, one isolate was resistant to INH. Moreover, It was found that all of the cases had pain and / or movement limitation, trauma in two cases, contact story with TB in one case. Chronic granulomatous inflammations characterized with caseification necrosis were detected in 3 cases. It is evaulated that the radiological findings of the cases were accordance with TB.
Conclusion: In the light of these findings, bone-joint TB might be a important reason of chronic bone-joint pains and movement limitations. We suggest that the isolation of the bacillus and determination of anti-TB drug resistance pattern with clinical findings, radiological and histopathological methods is important for the diagnose.

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Unusual pediatric tuberculosis case study highlights the need for better monitoring tools

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Introduction: *M. tuberculosis* infection causes a granulomatous immune reaction and a typical tissue necrosis called "caseous necrosis." Urogenital TB is rare in children. Genitourinary tract becomes involved by tuberculosis through hematogenously implanted bacilli in the kidney, either during the primary infection or later. Often those primary foci remain dormant for varying periods of time.
Case report: A child, 13 years old, was admitted to the hospital for severe cough, abdominal pain, weight loss and fever. Chest x-ray was normal. Abdominal computed tomography (CT) scan revealed hydronephrotic transformation of the left kidney and calcification (0.38 cm) in the upper portion of kidney. The initial diagnosis was kidney stone disease. *Mantoux* test resulted in 15 mm of induration, and QuantiFERON®-TB Gold test was positive. Low dose chest CT revealed calcified bronchopulmonary and subcarinal lymph nodes, and few miliary nodules in lung parenchyma. Urine sample was acid-fast bacillus testing-positive. *M. tuberculosis* cultures were obtained from both urine and bronchoalveolar washing samples. Final diagnosis: urogenital, miliary tuberculosis.
Contact investigation: revealed that child`s father was diagnosed with tuberculosis when the child was one year old indicating the history of exposure. The father was successfully treated; however, he deceased two years later of different cause. Both father`s and child`s *M. tuberculosis* isolates were drug-susceptible to the first-line drugs isoniazid, rifampicin, ethambutol and second-line drug ofloxacin tested in the Bactec MGIT 960 liquid culture and drug susceptibility testing system. Genotyping results revealed identical spoligotype SIT53 and IS*6110* pattern with five bands for both patients. These results indicated the high possibility of infection link.
Conclusions: This case report highlights the possibility of reactivation of latent TB infection in child after 12 years post-exposure, difficulties in diagnosis of urogenital TB, and importance of appropriate evaluation and management after TB exposure to prevent disease in future. The risk of reactivation of latent tuberculosis from calcified granulomatous lung lesions should be considered.
Acknowledgements: This study was supported by Latvian National Research Programme VPP 5.7. "BIOMEDICINE"

P 135

Pediatric tuberculosis in indigenous population in Puerto Nariño (Amazonas), Colombia

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Introduction: Pediatric tuberculosis (Pediatric TB) is an understudied key global public health problem. In 2015, Colombia had 281/12 863 023 cases in children younger than 15 years (incidence: 2.3/ 100 000). TB in indigenous population corresponded to 689 cases of whom 11.9% were in children younger than 15 years. Amazonas department presented the highest incidence in the country (72/100000). Our objective was to characterize clinically and epidemiologically indigenous patients younger than 15 years diagnosed with pulmonary tuberculosis in Puerto Nariño (Amazonas),

Colombia
Methods: We studied children younger than 15 years with respiratory symptomatology of any duration, recording clinical issues relevant to active TB through a medical questionnaire and consultation. We performed serial induced or spontaneous sputum collection for acid-fast staining, culture and phenotypic/ genotypic drug susceptibility testing.
Results: We examined 2550 indigenous children. 9.1% of children (33/363) were TB-positive, 66% were men, 69.7% had 5-14 years and the majority came from rural areas (81,8%). Among TB cases, 90% of sputa were induced, 18.2% had positive BK and 100% were culture positive. Mean cough duration was 9 days, mean expectoration duration was 7 days, 27.3% of children had no expectoration, 33% had previous TB contact, 12.1% lived with current TB cases, 1 patient had previous TB diagnosis, 69.7 % lived in overcrowding dwellings, 48.5% had BCG scar, 23.3% had abnormal auscultation and 12.1% showed adenopathies. We found 1 MDR-TB case.
Conclusions: Pediatric TB incidence (1 294/100 000) was 563 times higher than in general pediatric population (2.3/ 100 000). Induced sputum was a good alternative for sputum sampling: it identified 90% of TB cases.

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Ten years of Pediatric Tuberculosis: A retrospective study in Mersin, Turkey

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Objective: The aim of the present study was to evaluate paediatric TB cases that were confirmed by culture positivity in our region, retrospectively.
Materials and Methods: A total of 1205 clinical samples of 518 suspected TB patients, sent from various pediatric clinics to the Mycobacteriology Laboratory of the Medical Microbiology Department of Mersin University Medical Faculty, were examined retrospectively, between January 2006 and December 2016. Patients with culture positivity were included in the study group. Patients data were collected according to demographic features (age, sex), close contact story with the TB patient, tuberculin skin test positivity, microbiological diagnostic methods, organ and tissue location.
Results: Of the 518 patients, 27 (5.2%) were determined as culture positive. Sample distributions as follows: Sputum, starvation gastric fluid, bronchoalveolar lavage, pleural fluid, tissue, abse, biopsy, cerebrospinal fluid, gaita. Of the 27 patients, 16 were culture positive, 11 were both culture and ARB positive. Of the 27 patients, 14 cases (51.9%) were pulmonary TB; 3 cases (18.5%) were TB meningitis; 2 cases (7.4%) were miliary TB, 2 cases (7.4%) were TB pleurisy; 2 cases (3.7%) were bony-articular TB; 2 (7.4%) cases were TB lenfadenitis; 1 (3.7%) case was intestinal TB and 1 (3.7%) case was both pulmonary TB and TB pleurisy. One of the TB lymphadenitis cases was detected as occurred due to the BCG vaccine strain. Of the 27 patients, 18 (66.7%) were male, 9 (33.3%) were female and the mean age was 10.1 (min-max: 5 months-15 years). The family contact history was detected in 13 cases and 10 cases had tuberculin skin test positivity.
Conclusion: The diagnostic approach in pediatric TB is usually based on history of TB contact, tuberculin skin test positivity, clinical and radiological findings rather than bacteriological examination. In the light of all these findings, correlations between these parameters, especially culture positivity in close contact story with the TB patients, might be crucial to the certain diagnose in pediatric TB.

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Efficacy of isoniazid in a syrup comparing to tablets in providing IPT in children

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Summary: The purpose of the study was to conduct an analysis of the efficacy of isoniazid in the form of syrup vs. isoniazid in tablets for providing IPT in children.
Materials and methods: 104 children (0-5 years old) who were in contact with MTB and eligible for IPT were divided into Main Group, n= 56, who received isoniazid in the form of syrup (10 mg\kg daily = 0.5 ml\kg), and Control, n=46, who received isoniazid in tablets (standard doses).
Results: Significantly lower levels of total protein were reported in Main Group - by 9.6% than in patients in Control ($p<0.05$). Also significantly higher levels of bilirubin - by 17.2 %, ALT (alanine

transaminase) - by 19 %, AST (aspartate transaminase) - by 22.4 % and thymol test - by 15.5 % were reported in Control compared with Main Group. ($p < 0.05$), evidencing that there are preconditions for the development of toxic hepatitis when using isoniazid in tablet form. It is well established that the changes in blood chemistry were much more pronounced in children who received isoniazid tablets and were increasing up to the completion of the course of chemoprophylaxis.

Conclusions: The results of complex clinical, laboratory and instrumental study allow us to recommend the syrup form of isoniazid to be a preferable drug for providing IPT in children.

P 17

Physiological impact of the evolution of the rpoB mutation

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Background: Bacilli within an infected lung cavitary lesion spontaneously evolve mutations that confer resistance and are subsequently selected following antibiotic treatment. During this evolutionary process both drug susceptible and drug resistant bacilli may be present. This mix state of susceptive and resistant bacilli captured at a distinct point in time may change during the course of infection and drug selection. The complexity of the population structure in each sputum sample may thus define the outcome of molecular and phenotypic drug resistance testing which in turn may determine how the patient will be treated. We hypothesise that the rpoB mutation will influence the transcriptome of the rifampicin mono-resistant isolate compared to the progenitor rifampicin susceptible isolate.
Methods: A sputum sample from an individual patient containing a heterogeneous population of both a rifampicin mono-resistant Beijing Ser531Leu clone and its susceptible progenitor was selected. DNA was extracted and sequenced using the Illumina HiSeq platform and analysed using an in-house bioinformatic pipeline. RNA was extracted and sequenced using the Illumina platform and analysed using Chipster, an open source bioinformatic platform.
Results: Whole genome sequencing identified two different variants unique to the rifampicin mono-resistant isolate (excluding rpoB mutation) and two unique variants belonging to the susceptible isolate. The majority of the differentially expressed genes were transcription regulators as well as small subset of sigma factors/anti-sigma factors.
Conclusion: The small number of variants between the two isolates suggests that resistant isolate evolved from the susceptible progenitor. Our comparative transcriptomic analysis showed that microevolutionary events within the rpoB gene had a considerable influence on transcription. Consequently, the expression of bacilli's stress response sigma factors and regulatory genes were down-regulated. This in turn led to a down-regulation of expression of a large number of genes, suggesting that the rifampicin resistant mutant has an altered physiology.

P 36

Description of the Population Structure and Genetic Diversity of Mycobacterium tuberculosis among Patients of Haitian Origin Living in Florida

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Background: Individuals of Haitian origin have one of the highest tuberculosis (TB) case rates in Florida. However, literature on the propensity of certain M. *tuberculosis* strains to transmit faster than background rate in this population is limited. We investigated the genetic diversity, occurrence of strain emergence, and determinants of emergent strains among Haitians living in Florida.
Methods: All culture confirmed TB cases reported to the Florida Department of Health (FDOH) are genotyped using spoligotyping and MIRU. We analyzed data on 482 TB cases of Haitian origin reported to FDOH from 2002 to 2014. We used the web application MIRU-VNTR*plus* for strain family and shared international types (SIT) assignment. We used SpolTools and the program *DESTUS* to asses train emergence, recent transmission index and genetic diversity. In multivariate regression, we measured the socio-demographic and clinical determinants of strain emergence.
Results: Of the 482 strains, 136 (28.2%) belonged to the Haarlem lineage, 130 (27.0%) to the LAM lineage and 91 (18.8%) to the T lineage. SIT50, H3 sub-lineage, was the most common spolygotype with 59 strains (12.2%). Sixty two spoligotypes were identified as orphans. The different isolates were characterized into 114 genotypes with average cluster size of 4.2, recent

transmission index and clustering rate of 0.76 and 0.24 respectively, and a virtual heterozygosity of 0.040. Adjusting for false discovery rate, The H3 sub-lineage, was identified as emergent (θ=46.84, p=2.44×10^{-04}, q=0.02785), i.e. spreading faster than background transmission rate. A history of incarceration (AOR=7.63, CI: 1.17, 49.79; p=0.0337) was the strongest predictor of strain emergence after controlling for age, gender, years in the US, HIV status, initial drug resistance, site of disease, illicit drug and alcohol use, and homelessness.
Conclusion: The H3 sub-lineage seems to be emerging in Haitian communities in Florida, largely driven by exogenous infection among individuals with a history of incarceration. Renewed efforts to track and treat TB patients of Haitian origin are warranted to curtail the spread of this *Mtb* clone.

P 37

Higher frequency of Mycobacterium tuberculosis ancestral EAI strains, in the Northern Region of Brazil, as compared to previous studies in South America and Brazil

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Background: Rare genotyping information is available for the *Mycobacterium tuberculosis* complex (MTBC) strains circulating in the Northern part of Brazil, including the state of Pará.
Methods: Thus, we characterized the MTBC clinical isolates from this state by spoligotyping and compare the data with patterns found around the world, besides analyzing drug resistance, and socio-demographic information. We also performed Geographic Information System (GIS) analyses to highlight potential specificities of MTBC strains distribution in the region using a panel of 980 strains obtained from pulmonary TB cases between 1998 and 2011 at Instituto Evandro Chagas and Lajboratório Central do Pará, Brazil.
Results: A total of 249 different spoligopatterns belonging essentially to evolutionary recent Euro-American lineages, as well as Central-Asian (CAS), Manu and ancestral East-African-Indian (EAI) lineages, were identified, in addition to strains with reportedly unknown lineage signatures. Regarding EAI lineage, the absence of spacers 4-9 and 23-24 suggested a close evolutionary relationship between such strains in Pará with those predominant in Mozambique, which might have contributed to the genetic diversity of MTBC strains in this region.
Conclusions: The most frequent lineages were Latin American Mediterranean (LAM), T, Haarlem and EAI. EAI lineage strains were found in a higher proportion as compared to previous studies from South America. Further investigations will help to achieve the optimal sustained TB surveillance and better disease management in this region of Brazil.

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Latent tuberculosis screening using T-Spot-TB test among people living with HIV: A retrospective study of 190 patients at a tertiary care hospital in Dubai

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Background: It is estimated that one third of the 33.3 million people living with HIV worldwide are infected with TB. Overall mortality is twofold higher for HIV/TB co-infected individuals compared to those with isolated HIV infection. Consequently, diagnosis of latent TB infection (LTBI) and provision of chemotherapy to those testing positive is strongly recommended according to several international guidelines despite the lack of a gold standard test to diagnose LTBI. This article reviewed the epidemiology of latent TB infection among adult people living with HIV following up in a tertiary care centre in Dubai, United Arab Emirates (UAE). The UAE is considered a low prevalence country for both HIV and TB. There are limited data from UAE on the performance of screening for latent tuberculous infection among people with HIV/AIDS.
Material/methods: A retrospective study included all HIV-infected patients who were screened for LTBI using T-SPOT.TB test at Rashid hospital, Dubai from January 2016 until December 2016, through medical records. Data was analysed for demographics, T-SPOT status, CD4+ T-cell level at time of testing, viral load and current antiretroviral therapy status. Patient with active TB either during study period or in the past were excluded.
Results: Two hundred and nine patients with HIV were evaluated during this study period and 190 were included in analysis. 52(27%) were female and 138(73%) were male. 140/190(74%) of patients were UAE national, 24/190(12%) were African and 15/190 were from other Arab countries. T-SPOT was reactive in 33/190(17%) of cases, 155/190(82%) were nonreactive and only 2/190 1%) patients had an indeterminate result. The observation that only 2/190(1%) of the tests done in our study produced indeterminate results is reassuring in this regard. There was no statistically significant association between low CD4+ T-cell count, viral load and T-SPOT reactivity. Over 70% of patients in both arms have suppressed HIV viral activity with viral load below 50 copies/ml. A CD4 cell count less 200 cells/mm3 was observed in less than 30% of cases in each group, whereas over 30% of patients in each group had a CD4 cell count of more than 500 cells/mm3. Twenty one (68%) patient with reactive T-SPOT were offered preventive therapy.
Conclusions: The study showed high compliance with international recommendations regarding the screening for LTBI in HIV infected patients in our centre. It also showed low prevalence of LTBI among our cohort of HIV infected patients and over two third of patients received prophylactic therapy. TB continues to be a major threat to any HIV control program and all efforts should be taken for early diagnosis and appropriate management.

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Classification and evolutionary pathway of East Asian lineage of Mycobacterium tuberculosis

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Due to the high prevalence and successfulness there were many attempts to classify strains belonging to *Mycobacterium tuberculosis* (Mtb) lineage 2 and to clarify their evolutionary pathway. At least, 8 different genotyping schemes were proposed by leading scientific groups, based on different genomic markers, such as NTF/IS*6110* (Mokrousov *et al*., 2005), regions of difference (Tsolaki *et al*., 2005), SNPs datasets (Rad *et al.*, 2003; Filliol *et al*., 2006; Mestre *et al*., 2011), VNTR and WGS (Coll *et al*., 2014; Luo *et al*., 2015; Merker *et al*., 2015). In this study, we investigated congruence of the phylogenetic classification methods of the Mtb lineage 2 using whole genome sequencing data of 1,398 strains.
The dataset consisted of 13 WGS studies was obtained from NCBI and ENA. SNPs calling was carried out using a standard pipeline (Bowtie 2-SAMtools-VarScan). After excluding the repetitive, PE-PPE-PE_RGRS, DR associated genes and artifactual SNPs linked to indels, we used remaining 39,786 SNPs to reconstruct a ML phylogeny of Mtb lineage 2. SpoTyping and ISMapper were used for *in silico* spoligotyping and analysis of the IS*6110* insertion in NTF region, respectively.
We superposed new schemes onto already long-used phylogenetic framework of the lineage 2 on the phylogenetic tree level and intersected SNPs, which were used by different authors. We confirmed that lineage 2 comprises 2 major clades, designated proto-Beijing, which harbors unusual 43-signal spoligoprofile, and Beijing, with well-known spoligoprofile. In its turn, Beijing clade is divided to ancient and modern groups, in which smaller groups can be clearly identified. For ancient Beijing we detected from 2 (Luo *et al.*) to 6 (Mestre *et al.*) such groups. Additionally we identified strains with mutation in *mutT2* and intact *ogt* gene that are transitional between ancient (*mutT2*(wt)/*ogt*(wt)) and modern (*mutT2*(mut)/*ogt*(mut)) Beijing. Due to star-shaped phylogeny,

relationships between modern Beijing strains were less clear than for ancient, and to a greater extent depended on the collection diversity.
In conclusion, we correlated newly discovered and "old" molecular markers of the lineage 2. In practical terms, we aimed to facilitate communication between different research groups. We sought to clarify the evolutionary pathway of this lineage, and in particular, to highlight its known epidemic clusters.
This work was supported by 17-15-01412 grant of the Russian Science Foundation of the Russian Federation.

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Epidemiological analysis of Mycobacterium tuberculosis complex strains isolated and tested for MIRU-VNTR in 2014-2015 in Lombardy region (North Italy)

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Introduction: Molecular typing of *Mycobacterium tuberculosis* complex (MTBC) strain is used to monitoring epidemiology of TB. The aims of the study were:
- Analyse different lineages isolated in 2014 and 2015
- Associate nationality of patients and lineages
- Evaluate the impact of migration towards Italy
- Monitor drug-resistant strains spread

Material and methods: All culture positive new TB cases diagnosed in Lombardy region (North Italy) from January 2014 to December 2015 were included. Culture and susceptibility test were performed on BACTEC-MGIT 960. Tests by HAIN were used to identify isolates and to confirm isoniazid and rifampicin resistance.
We have performed 24 loci MIRU-VNTR analysis on DNA extract of each MTBC strain with MIRU typing kit (Genoscreen™) to determine lineages. Patients data were from regional register for infection diseases.
Results: During two-years 1416 MTBC strains were collected (702 in 2014 and 714 in 2015).
In table are shown the results.
MDR and XDR strains accounted for 3,27% and 2,80% respectively in 2014 and 2015.

Conclusion: Our results show that there is no difference about distribution of lineages between 2 years. In 2015 we observed an increase of strains from East Europe. We have seen a small decrease of drug resistance in 2015. There is no significant evidence of trasmission from foreignes to italians.
Despite a new generation of methods for MTBC typing, MIRU-VNTR remains a suitable tool for epidemiological analysis.

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Genetic and biological diversity of the Mycobacterium tuberculosis East African Indian lineage

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The impact of the genetic variance of *Mycobacterium tuberculosis* complex (MTBC) strains on the outcome of tuberculosis (TB) infection has been neglected for decades. Based on classical genotyping methods like 24-loci-MIRU-VNTR typing as well as genome based studies more than 20 major MTBC genotypes/lineages have been defined so far during the last 15 years. However, linking strain diversity to pathobiological characteristics such as transmissibility or resistance rates is still challenging and only described in few studies. Especially variances within a phylogenetic lineage like the East African Indian (EAI) population (lineage 1) have not been investigated in detail so far. We analyzed 170 clinical EAI isolates from different geographical settings using the Illumina Next Generation Sequencing (NGS) approach to allow a high resolution investigation of the EAI population structure. NGS data were mapped to the sequence of the reference strain *M. tuberculosis* H37Rv (NC_000962.3) to identify single nucleotide polymorphisms (SNPs) as well as insertions and deletions (indels). Overall, 4 major phylogenetic groups/sub-lineages could be defined from which 14 representatives were further investigated. $^{1}H,^{13}C$ – HSQC NMR based lipid profiling revealed sub-lineage

specific differences in the phenolic glycolipid (PGL) pattern, which has been associated with mycobacterial hyper-virulence in previous studies. While growth analysis of these strains in human macrophages showed no group specific differences further analysis of the pathobiological consequences of the lineage specific PGL patterns are currently under investigation.

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Few genetic clusters are involved in the transmission of Mycobacterium tuberculosis with discordance between Xpert MTB/RIF and MGIT assays for rifampicin resistance in Sao Paulo, Brazil

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Introduction: Since the implementation of the Xpert MTB/RIF assay (Cepheid, France) in the state of Sao Paulo, Brazil, discrepant rifampicin susceptibility results have been observed between that genotypic assay and phenotypic susceptibility testing by BACTEC MGIT.
Objective: This study aimed to evaluate the association between *M. tuberculosis* (MTB) clusters and specific mutations on *rpo*B gene of phenotypically rifampicin-susceptible cultures from clinical samples yielding rifampicin-resistant results.
Material and Methods: All isolates received in our laboratory since October 2014, exhibiting rifampicin resistance by Xpert MTB/RIF but rifampicin susceptibility by MGIT (1.0 mg/L rifampicin) are being sequenced in the predominant rifampicin-resistance-determining region (RRDR) of *rpo*B gene and also analysed by *IS*6110 restriction fragment length polymorphism (RFLP) using Bionumerics software (version 7.5).
Results: To date, 74 of 82 isolates (one per patient) had the RRDR region of the *rpo*B gene sequenced and their lineages characterized. Fifteen distinct kinds of mutations were observed among 59 isolates and none was observed in 15 isolates. The most common mutations were H526N (n=26; 44%) and one silent mutation (TTC>TTT) at codon 514 (n=16; 27%), followed by L511P (n=4; 7%), L511R and L533Q (n=2 each; 3%). The remaining nine isolates carried one (2%) of the following *rpo*B mutations: T508A/G/T, D516F/Y, L524L, H526S, S531L, and L533P. Nine of these types of mutations, harboured by 36 (49%) isolates, presented well documented association with rifampicin resistance.
Genotyping showed that 54 (73%) isolates were clustered into nine groups. The four dominant clusters (SP2ga, SP25, SP25a and SP5o) comprised 51% (n=38) of isolates. Eighteen out of 19 isolates belonging to the SP2ga cluster and all seven of the SP5o cluster carried *rpo*B H526N mutation, comprising 25 of 26 isolates carrying that mutation. Isolates clustered into clusters SP25 (n=5) and SP25a (n=7) harboured a silent mutation at codon 514 (C>T), comprising 12 out of 16 *rpo*B F514F silent mutants.
Conclusions: The high percentage of clustered MTB isolates could suggest that a small number of clusters are causing the transmission of a large part of MTB showing discordance between phenotypic and molecular rifampicin susceptibility testing.

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The epidemiology of M.tuberculosis in Riga region, Latvia: comparison of three molecular genotyping methods

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Introduction: Molecular genotyping methods of Mycobacterium tuberculosis (MT) play an important role both in clinical studies of tuberculosis and in the epidemiological

investigations. On PCR-based methods such as spoligotyping and mycobacterial interspersed repetitive unit variable-number tandem repeat typing (MIRU-VNTR) are rapid, easy performed, and can be done with very small quantities of crude DNA. On the other hand, restriction fragment length polymorphism analysis of IS6110 (IS6110-RFLP) have been previously known as the gold standard for MT strain typing, but is time-consuming and needs good quality, purified and concentrated DNA. However, all these methods have their own particular advantages and they differ with their discriminatory power.

The aim: of this study was to characterize the diversity of MT isolates circulating in Riga region, Latvia, and to evaluate the discriminatory power of three methods used for molecular genotyping of MT.

Material and methods: MT positive cultures were isolated from different specimens of TB positive patients living in the city of Riga and its surroundings from January 2009 till February 2012. Molecular genotyping using spoligotyping, 24 loci MIRU-VNTR and IS6110 RFLP methods was performed for MT DNA isolates obtained from cultures grown on Löwenstein Jensen slants.

Results: A total of 299 M.tuberculosis DNAs were genotyped. 74 different spoligotypes were identified by the spoligotyping method. Genotypes belonged to East-Asia (25%, n=76), Euro-American (63%, n=188) lineages; 12% (n=35) showed unpublished SIT. By the MIRU-VNTR analysis, 173 different patterns were identified, including 39 clusters. IS6110 RFLP showed 196 different patterns, including 35 clusters.

Conclusions: This study highlighted the high genetic diversity of MT strains in Latvia. Among the three methods, spoligotyping showed the lowest discriminatory power (0.9049) compared to the other two techniques, however, was fast and well adopted for clinical needs. IS6110 RFLP showed the highest discrimination power in our study, especially for Beijing strains. In combination with detailed epidemiological data IS6110 RFLP is helpful for the in-depth understanding of epidemiological processes in settings where the Next-Gen sequencing is not available as a routine method.

Acknowledgements: This study was supported by the EU 7th Framework TB- PANNET and Latvian National Research program VPP “BIOMEDICINE”

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Characterization of Mycobacterium tuberculosis strains isolated from pulmonary tuberculosis patients in the state of Khartoum, Sudan using Line Probe Assay and Spoligotyping

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The aim of this study was to characterize the lineages of *Mycobacterium tuberculosis* isolated from pulmonary TB patients in Khartoum state-Sudan.

Background: The Republic of Sudan is one of the largest countries in Africa, located in east Africa with links both to the Arab world and Sub Saharan Africa. It is sharing borders with many countries in the region, which allows free movement of citizens from different countries across the borders of Sudan.

Reports from 2012 estimated incidence of TB in Sudan to 114/100,000. In this study, DNA from a total of 120 Mtb strains isolated between 2007 and 2009 were sent to WHO SRL for EQA purposes from the NRL in Khartoum, Sudan.

Methods: Sputum samples from smear positive patients was collected consecutively from selected TB diagnostic centers in Khartoum state during the period 2007-2009. At the NRL species identification was done using biochemical and DNA based methods. Drug susceptibility testing (DST) was done using the proportion method on LJ-medium. Confirmation of phenotypic DST results was done by LPA to detect mutations in resistance related genes. DNA samples from 60 MDR and 60 non-MDR isolates were examined by LPA as part of the EQA at the WHO SRL in Stockholm. Molecular epidemiology analysis was done using Spoligotyping to determine the genetic diversity of the isolates circulating in this region of Sudan.

Results: A total of 120 DNA samples were studied using LPA and Spoligotyping at the SRL in Stockholm. Comparison of LPA and phenotypic DST results showed 100% agreement for non-MDR and 60% for MDR samples. Spoligotyping revealed a total of 112 patterns from which 6 patterns represented clustered isolates with a total of no less than 105 (88%) of the strains. The CAS1 Delhi/family was the predominant type detected in 61 isolates (51%), where 25 of the strains were MDR and 36 were susceptible. It was followed by H3/family with 19 (16%) strains, and 11 strains with LAM3/family, 14 strains with T2/T1/family type and 2 strains each with Beijing

type and S family.
Conclusion: The observation obtained using spoligotyping from the Sudanese patients reflects in comparison to the global distribution highly diverse clades circulating in the country. In general the study could not detect any major differences between MDR and non-MDR isolates within the clade types.

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Investigation of Pyrazinamide Resistance in Drug Resistant Mycobacterium tuberculosis complex in Turkey

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Objective: Rifampicin (RIF), isoniazid (INH), pyrazinamide (PZA) and ethambutol (EMB) are the first-line drugs in the treatment regimen of TB which remains an important global health problem. Considering the unique ability of PZA to eradicate persistent bacilli and decrease the treatment process from 9–12 months to 6 months, it's obvious the PZA forms a critical cornerstone of this regimen. The aim of the present study was to determine the incidence of PZA resistance in drug resistant M. tuberculosis complex (MTC) isolates in our region.
Materials and Methods: A total of 57 MTC isolates resistant to at least one of the RIF, INH, EMB and streptomycin (SM) anti-TB drugs, which were isolated in the Mycobacteriology Laboratory of Medical Microbiology, Department of Mersin University, Faculty of Medicine included in this study. PZA sensitivities were determined with using the BACTEC MGIT 960 sensitivity test method (accepted as standard method by CLSI). The susceptibility test was performed using the BD MGIT 960 PZA Kit, PZA Medium (Becton Dickinson, Sparks, MD) and the critical concentration of 100.0 µg/ml PZA according to the manufacturer recommendation. İdentification of MTC isolates were determined by spoligotyping. M. tuberculosis H37Rv (PZA sensitive) and M. bovis BCG (PZA resistant laboratory isolate) were used as the control strains in the study.
Results: Of the 57 MTC isolate, PZA resistance was determined in 6 (10,5%) isolate. One of the PZA resistant isolate was identified as M. bovis BCG by spoligotyping. PZA resistance was detected in 5 isolates (2 isolates with EMB, 2 isolates with INH and 1 isolate with SM). In Additionally, PZA with INH and EMB resistance was detected in the M. bovis BCG strain. PZA resistance was not detected in MDR-TB isolates.
Conclusion: In our country, because of the PZA resistance was not routinely tested in tuberculosis laboratories, the data of PZA resistance rate is not known exactly. Therefore, a nationwide sensitivity test policy can be developed for determining the PZA resistance rates with the increase of further similar researches.

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First study of the nationwide population structure of the Mycobacterium tuberculosis complex in Benin

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Background: Genotypic diversity studies are important for deeper understanding of circulating *M. tuberculosis* complex (MTBc) strains in a population. To date, no study has investigated the nationwide population structure of the MTBc in new tuberculosis (TB) patients in Benin.
Methods: Smear-positive sputa were collected from a representative sample of new TB patients from all regions in Benin, through a prospective nationwide surveillance. The next four new TB patients diagnosed after a retreatment patient were included. DNA was extracted from the sputa using the Maxwell DNA Tissue Purification kit, after a digestion with proteinase K. Spoligotyping was performed using standard methods. The TB lineage database was used for lineage assignment, and SITVIT web database for SIT-type assignment.
Results: From 240 patient's sputa from all regions of Benin, the majority (56%) came from Atlantique/Littoral. A spoligotype pattern was available for 234 (98%) of the specimens.
Nationwide, Lineage (L) 4 (Euro-american) strains were the most prevalent (47.9%, 112) followed by L5 (*M. africanum* West African 1) (73, 31.2%), L6 (*M. africanum* West African 2) (21, 9%), L2 (East-Asian Beijing) (12, 5.1%), L1 (Indo-oceanic) (12, 5.1%,), and L3 (East African Indian) (1, 0.4%). Three *M. bovis* strains (1.3%) were also detected. The prevalence of *M. africanum* L5 and L6 combined was 40.2%.

The comparison of the prevalence of predominant lineages (L4, L5, L6) showed that the distribution of lineages differed significantly across regions (Fisher exact p=0.027). In Borgou/Alibori, L6 was most prevalent, at 45.5% (5/11), versus 3.2%-15.4% in other regions. L5 strains were most prevalent in Mono/Couffo at 43.5% (10/23) versus 0%-35.5% in other regions.
From the 106 different spoligotypes found, 62 (58.5%) were not yet described in the SITVIT web database. There were 22 clusters of 2 to 54 individuals, including 2 clusters from new spoligotypes. Overall, the most prevalent spoligotype was SIT 61 (23.1%), followed by SIT 53 (5.6%).
Conclusion: There is a great diversity in the MTBc strains from new TB patients in Benin, with many new genotypes. L4 was the most prevalent, followed by *M. africanum* L5 and L6, and the distribution of lineages differed significantly across regions. Further studies based on whole genomes must be conducted in order to understand when different strains became endemic in the different regions.

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Molecular characterization of Mycobacterium tuberculosis clinical isolates from TB patients in Estonia

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Objective: This study investigated the currently circulating *Mycobacterium tuberculosis* genotype families and clones in Estonia, also in comparison with earlier studies carried out 20 years ago. We hypothesized that *M. tuberculosis* population in Estonia was influenced: (i) by massive human influx from Russia in 1945-1991; (ii) by interaction within EU after the country regained its independence in 1991.
Materials and methods: *M. tuberculosis* strains from all consecutively enrolled in 2014, newly-diagnosed 92 TB patients were studied. All patients were from North Estonia and were without proven epidemiological links (12 HIV-positive). Beijing genotype was detected by PCR of the *dnaA-dnaN*::IS*6110* insertion. All non-Beijing strains were subjected to spoligotyping; the profiles were compared to SITVIT_WEB. LAM family was detected based on *Rv0129c* SNP; LAM isolates were tested for RD115, RD174, RD-Rio, and LAM-RUS markers. Beijing B0/W148 cluster was identified based on *Rv2664-Rv2665*::IS*6110* insertion.
Results: The 40.2% (37 of 92) *M. tuberculosis* strains were assigned to the Beijing genotype; Beijing B0/W148-cluster was identified in 59.5% (22/37) Beijing strains; all B0/W148 strains were drug-resistant. Multidrug resistance (MDR) was found in 22.8% (21/92) strains and only in Beijing genotype strains. MDR was more prevalent among Beijing B0/W148-cluster strains (81.8%; 18/22) compared to other Beijing (20.0%; 3/15; P=0.0007).
Among 55 non-Beijing strains, 30 spoligotypes (SIT) of different genetic lineages were identified: T (16.3% of all collection), LAM (12%), Ural (11%), Haarlem (10%), X (2%) and unclassified (3%). Four isolates had new spoligotypes not found in SITVIT_WEB. One isolate (SIT326) was defined as *M. africanum*. The 14.5% (8 of 55) non-Beijing strains were resistant to SM (n=5), INH, RIF, and SM+INH (n=1 each).
Conclusions: The Beijing genotype included all MDR *M. tuberculosis* strains in this study and most of them (18/21) were B0/W148 cluster. Compared to the countrywide Estonian studies carried out in mid-1990s (Kruuner et al., 2001; SITVIT_WEB/T. Koivula), the prevalence of the Beijing genotype increased and that of B0/W148 increased significantly. Trends for major non-Beijing genotypes were opposing but non significant (slight decrease for LAM, increase for Haarlem); however an increase of Ural MDR sublineage should be noted.
Acknowledgements: Russian Science Foundation (project 14-14-00292).

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Investigation of the changes in incidence and resistance rates of Mycobacterium tuberculosis in the last 6 years of Cukurova, the strategic region of Souther Turkey

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Tuberculosis is the second leading cause of death from an infectious disease worldwide and still one of the major public health problems of Turkey. Rapid diagnosis, detection and following the spread of drug resistance and epidemiological investigation of *M. tuberculosis* complex is play a big roll in the success of treatments and control programs.

As it is known, the Cukurova region receives considerable immigration in recent years due to its strategic location. In particular, immigrants coming from high TB incidence countries can represent a new epidemiological issue in the TB care and control.
This study aimed to investigate the changes in incidence and resistance rates of *M. tuberculosis* in the last 6 years of 8 important city of Southern Turkey that are characterized both by a high TB incidence and population size.
A total of 67.106 sputum collected from patients who were admitted to cities dispensaries between 2011-'March 2017 were sent to Region Tuberculosis Laboratory for analysis. All specimens were inoculated on Lowenstein-Jensen and BACTEC-MGIT 960 after decontamination and direct preparations stained with acid fast strain method were evaluated microscopically. *M. tuberculosis* complex and mycobacteria other than tuberculosis were differentiated by Immunochromatographic card test and the susceptibility testing for the MTC strains to primary antituberculosis drugs were performed by BACTEC-MGIT 960 system in region tuberculosis laboratory.
According to the results of our study, the positivity and resistance rates according to years are as follows; positivity rate (PR) %4,5 and MDR (MR) rate %6.4 in 2011, positivity rate (PR) %7,2 and MDR (MR) rate %8,8 in 2012, positivity rate (PR) %8,7 and MDR (MR) rate %5,8, in 2013, positivity rate (PR) %10,2 and MDR (MR) rate %4,3 in 2014, positivity rate (PR) %9,5 and MDR (MR) rate %1,3 in 2015, positivity rate (PR) %9,5 and MDR (MR) rate %1,2 in 2016. And also the increase in foreign nationals in the last 2 years is also remarkable totally 1067 clinical sample with positivity rate %13,46. Analysis of 2017 samples is not yet completed, work continues.
The most remarkable thing in the result of the study; there has been decline in multi drug resistant strains, despite an increase in the annual positive rate. Our study is ongoing.

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Drug resistance patterns of phylogenetic sublineages within Mycobacterium tuberculosis Beijing genotype strains in West Siberia, Russia

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Background: The Siberian region of Russia is characterized by a significant level of tuberculosis prevalence, incidence and mortality. The local population of *Mycobacterium tuberculosis* is dominated by Beijing family prevalent at ~60%. The city of Omsk in West Siberia is the second most populated city in the Siberian region. The Omsk region borders the northern part of the Republic of Kazakhstan, where the proportion of Beijing genotype strains is very high (>85%) and a cross-border human (and pathogen's) exchange is frequent. This study investigated drug resistance properties of *M. tuberculosis* Beijing genotype strains in the Omsk region.
Methods: We studied *M. tuberculosis* isolated from epidemiologically unlinked TB patients from Omsk region in West Siberia, Russia in 2015-2016. Beijing genotype was detected by testing specific IS6110 in dnaA-dnaN locus. Beijing genotype strains were subdivided into ancient and modern sublineages based on analysis of RD181 deletion (intact in early ancient Beijing) and NTF locus analysis (intact in modern strains). Successful Russian cluster Beijing B0/W148 as detected by testing specific IS6110 in Rv2664-Rv2665 intergenic region.
Results: The study collection included 129 *M. tuberculosis* isolates of the Beijing genotype (62% of the entire collection). Almost half of the Beijing strains were MDR (51.2%, 66/129). Beijing B0/W148 cluster was detected in 21.7% (28/129) of Beijing strains; of them 14.3% (4/28) were polyresistant and 85.7% (24/28) were MDR. Other, non-B0/W148 strains of the modern Beijing sublineage were detected in 61.2% (79/129) of all Beijing strains; while 51.9% (41/79) were susceptible and 27.8% (22/79) were MDR. Strains of ancient and early ancient Beijing sublineages were found in 17.1% (22/129); this may be explained by close geographical proximity to Central Asia. Both ancient and early ancient Beijing strains were dominated by MDR strains: 88.9% (16/18) and 100% (4/4), respectively.
Conclusions: *M. tuberculosis* population of the Beijing genotype in the Omsk region in West Siberia is dominated by strains of the modern sublineage of this genotype family. At the same time, high level of drug resistance, including MDR, was found in strains of both recently emerged epidemic cluster B0/W148 and evolutionarily ancient sublineages.
Acknowledgement: We acknowledge funding from Russian Foundation for Basic Research grant #17-04-00367.

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Rapid detection of IS6110 insertions in NTF-1 region, a first step toward high-throughput assays for molecular diagnostic and classification inside Beijing lineage (lineage 2) using microsphere-based flow cytometry

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W strain is a famous subgroup of Beijing lineage, responsible for one of the first MDR outbreak in New-York city in the 90ies, and characterized by two tandem insertions of IS6110 around NTF-1 region (Plikaytis *et al*., 1993). Number of IS6110 insertions in NTF-1 locus has then proposed to subclassify Beijing isolates.
We aimed here to 1) clarify Beijing lineage classification by reconstituting a phylogenetic tree positioning most used classification markers, specifying all family names used for classification and positioning famous strains such as W strain; 2) develop a straightforward method to detect IS6110 copies in the NTF-1 locus by using the Luminex® technology.
Based on published WGS of 5239 isolates (see Shikitov et al, poster ESM 2017), we derived a phylogenetic tree that we annotated with all markers included in the 8 most used classifications. This approach clarifies for instance that "modern Beijing" (defined by the presence of a single insertion in RD105/RD207/RD181 and mutations in *mutT2*, *mutT4* and *ogt* genes.
To develop a method on Luminex200® system to detect IS6110 insertions in the NTF-1 locus, we used an American collection of 48 strains including 18 W outbreak isolates, 19 non-outbreak "modern Beijing" (one IS6110 copy at the 3' end of NTF-1 locus), and 11 strains without any copy of IS6110 at the NTF-1 locus. We detected: 1) 18/19 strains with a 3' IS6110 insertion at the NTF-1 locus; 2) 15/17 carrying two insertions; 3) 11/11 with no IS6110 insertion in NTF-1. This method (to be validated on an independent sample) helps distinguishing ancient Beijing, modern Beijing isolates and, among Modern Beijing, W strains.
We plan to use multiplexing capacities of Luminex200® systems to combine this new method with drug resistance mutations as developed in the TB-SPRINT technique. This combined method will help monitoring drug resistance spreading in regions with high Beijing isolates prevalence and/or suspicion of drug-resistance. Development of other, more general high-throughput tests using SNPs along major branches of Beijing phylogenetic tree are in progress to help subclassifying any Beijing isolate.

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Light microscopy for diagnosis of pulmonary tuberculosis in the eastern part of the Sudan

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Background: In the Sudan, estimated tuberculosis (TB) incidence was 66 per 100,000 in 2015 and TB diagnosis largely relies on clinical symptoms and smear microscopy. The specificity of smear microscopy is between 98-99% when used for clinical diagnosis and TB prevalence is usually >15% in the context of passive case finding in low resource countries. As a result the positive predictive value of a positive smear is high (90%) in this setting.
Aim: This study aimed to investigate the positive predictive value of a positive smear in patients investigated for TB in the eastern part of the Sudan.
Method: Two sputum samples of patients presenting with symptoms suggestive of pulmonary TB between June to October 2014 and January to July 2016 were investigated using light microscopy. Smear positive samples were stored at -20 degrees and sent to the German National and Supranational Reference Laboratory where all underwent repeat microscopy and culture. A real-time PCR was performed using the stored sediment to investigate any samples where the culture remained negative or was contaminated.
Results: A total of 383 samples were investigated. Repeat microscopy categorized 123 (32%) as

negative, among which 26% were culture positive and increased to 72% when PCR and culture results were taken into account. A total of 172, 16 and 9 samples were culture positive for *M. tuberculosis*, *M. intracellulare* and mixed cultures of different species. Furthermore, 27 samples had evidence of non-tuberculous mycobacteria (NTM) either by culture or PCR and 33 samples tested by PCR did not detect any evidence of mycobacterial DNA. Overall, 16% of samples had no evidence of *M. tuberculosis* using culture and PCR, resulting in a positive predictive value of 84% of smear microscopy in this setting.
Conclusion: Transport conditions need to be carefully reviewed when sending samples for culture, as a high proportion of samples was culture negative despite the evidence of mycobacterial DNA. Patients with NTM infection or colonization might be wrongly diagnosed with TB.

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Molecular characterization of Mycobacterium tuberculosis that causes active disease in indigenous people of Colombia

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Introduction: The indigenous peoples of Colombia because of their sociocultural characteristics are part of the vulnerable population with greater susceptibility to tuberculosis. Previous studies in Colombia have shown incidences of up to 500 cases per 100,000 inhabitants in this population, with the national incidence for the general population of 25, 6 cases per 100,000 inhabitants.
Objective: To characterize molecularly clinical isolates of *Mycobacterium tuberculosis* that cause active tuberculosis, circulating in indigenous people of Colombia between the years 2009 and 2014. **Methodology:** This is a descriptive, retrospective study in which 234 *M. tuberculosis* isolates from 229 patients were characterized molecularly by Spoligotyping and MIRU-VNTR. These isolates were part of a biologic collection of the Mycobacteria research group at the National Health Institute of Colombia (INS), obtained through routine surveillance during the period of 2009-2014. Socio-demographic variables of the population as well as first-line drugs susceptibility patterns were taken into account to determine significant possible associations with the described genetic families.
Results: 41 groups of indigenous people were identified. The Wayúu (13.10%), Embera Chami (11, 35%) and Awa (7, 9%) people had the greatest number of cases of tuberculosis, besides, 12 cases of MDR were found. Using Spoligotyping, 102 genotypes were identified (47.06% described in the database SpolDB4 and 52.94% orphan genotypes), which were organized in 30 groups (HGDI 0.9635). On the other hand, with the MIRU-VNTR methodology 230 genotypes were identified out of which 226 were unique patterns and 4 were grouped (HGDI 0.99985). After combining Spoligotyping and MIRU, 231 genotypes were found and these comprise in 228 unique patterns and 3 groups (HGDI 0.99988). The Sublineages for spoligotyping found were LAM (37, 18%), Haarlem (15, 8%), T (8, 1%), U (3, 2%), S (2, 6%), X (2, 1%) and Beijing (0, 85%), and High proportion of orphan patterns (30%). It is located for the first time in the country a genotype Beijing like SIT 406. Conclusions: The presence of 3 TB transmission cases were confirmed through the two genotyping methodologies in indigenous people studied. Additionally, 4 cases of exogenous re-infection and 1 case of mixed infection were identified. 50 orphan genotypes may be autochthonous to Colombian indigenous populations.

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Prevalence of Turkey's major spoligotype-based families; T1 and TUr and their association with drug resistance in South of Turkey

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Tuberculosis (TB) is one of the most important health problem caused by *Mycobacterium tuberculosis* complex (MTBC). The number of multidrug-resistant tuberculosis (MDR-TB) cases has increased in recent years. Definition of

*M*ycobacterium *tuberculosis* complex genotypes by spoligotyping and make correlation between drug resistant pattern can help us to track possible resistant genotypes and manage these patients to prevent to spread of TB. Previous studies done in Turkey showed that the major families of *Mycobacterium tuberculosis* strains were either T1 or TUR ,high phylogeographical specifity for Turkey, spoligotypes. In this study, our objective was to investigate prevalence of these major spoligopatterns of MTBC strains isolated from eight different cities of Southern Turkey and their possible association with drug resistance.

A total of 175 MTBC strains were randomly selected from isolated patients samples collected between 2013-2016 in eight cities from south of Turkey (Adana, Mersin, Osmaniye, Hatay, Gaziantep, Kilis, Kahramanmaras, Adiyaman). Cultures and resistance profiles were performed on BACTEC-MGIT 960 at the Tropical Disease Research and Application Center, Adana Regional Tuberculosis Laboratory. Distribution of MDR and non-MDR strains were 97 and 78 respectively based on phenotypic DST results. DNA was extracted from liquid media culture (MGIT) using acid washed mini-glass beads in Mickle cell disruptor. DNA extracts were analyzed by spoligotyping. SITVITWEB tool was used to determine lineages.

In total of 58 distinct spoligopatterns were identified among 168 interpretable spoligotype profiles. According to spoligotyping method, T1 and TUR families were considered as major groups with 80 (47.6%) and 42 (25%) sample numbers respectively. SIT53 (31.8%, n=51) and SIT41 (41%, n=39) were identified as common groups.

Of the analyzed isolates, the most prevalent clades were T1 (44.3%, n=43) and TUR (26.8%, n=26) to MDR-TB; as well T1 (47.4%, n=37) and TUR (20.5%, n=16) to non-MDRTB. Geographical distribution of these clusters widespread in Adana and Hatay with 36 (21.4%) and 17 (10.1%) for T1 followed by TUR with 20 (11.9%) and 9 (5.4%) strains.

Our results showed that we defined same predominant families and genotypes which were described in pervious studies conducted in different parts of Turkey. The sample numbers should be increased to get more significant correlation rates between clusters and resistance patterns for our region.

P 32

Non-tuberculous Mycobacteria isolated from Russian tuberculosis patients over a one year period

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Non-tuberculous Mycobacteria (NTM) can cause a severe lung disease in human including tuberculosis (TB) patients, especially in high TB burden settings where mixed Mycobacterial infections are known to occur often. The aim of the study was to estimate rates of NTM infection in TB patients in Samara region, Russia over a one year period.
Materials and methods: Commercially available GenoType® CM and AS kits (Hain Lifescience®, Nehren, Germany) were used for the identification of strains grown on solid and liquid media from pulmonary and non-pulmonary material of TB patients (both new and re-treatment cases) routinely tested at the Regional TB laboratory (Samara, Russia) in 2016.
Results: Out of 11032 positive cultures 111 strains isolated from 67 patients (44 men and 23 women) were identified as NTM. More often NTMs were isolated from men from 30-39 and 50-59 (16 and 11 strains respectively) and women from 30-39 and 60-69 age groups (7 and 5 strains respectively). An NTM strain was obtained only once from 50 (74.6%) patients, 2 strains – from 9 patients (13.4%), 3 strains – from 5 patients (7.5%); 1 patient (1.5%) produced 6 strains and another one – 7 strains of *M. kansasii* while 1 patient produced 15 strains of *M. avium* during the year. Eleven patients out of 67 received treatment at the TB Service for more than 1 year. Overall, molecular assays allowed to identify 7 different species of NTMs: *M. avium* (36 strains), *M. kansasii* (30), *M. fortuitum* (16), *M. intracellulare* (7), *M. gordonae* (6), *M. abscessus* (5), and *M. genavense* (1). Three strains were identified as a mixture of Mycobacterial species. Species for 3 more strains was not identified by molecular assay while another 4 strains were identified as high GC-containing bacteria.
Conclusion: TB patients in Samara, Russia have pulmonary infections caused by various NTM species. Rapid and precise identification using commercial PCR-based assays should be implemented into routine diagnostics especially in high TB burden resource-constrained settings where whole genome sequencing and other high-throughput techniques are still unavailable.

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Disseminated Mycobacterium Avium Complex and Disseminated salmonella group D coinfection in an immunocompetent middle aged patient: A case report

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Background: Mycobacteria other than Tuberculosis (MOTT) or non-Tuberculous mycobacteria (NTM) are used interchangeably to describe this group of ubiquitous organisms that are capable of inducing lung disease in different patient population. However, disseminated or extra-pulmonary manifestation of the infection has been mostly described as Mycobacterium Avium Complex (MAC) in patients with advanced HIV. A number of case reports described disseminated MAC infection mimicking malignancy in relatively immunocompetent hosts and some have linked it to underlying defects in IFN- ?? and IL-12 signaling pathways. This could lead to concurrent infections with salmonella group D as well.
Material/methods: Herein we describe a case of disseminated Salmonella group D and disseminated MAC coinfection in a relatively immunocompetent middle-aged female patient.
Results: A 49-year-old East Asian female presented to our hospital with acute history of epigastric pain, right iliac fossa pain , burning on micturition and was worked up for Acute Pyelonephritis. Urine and blood culture confirmed the growth of Salmonella Group D infection and

she was managed accordingly. As part of work up, she underwent CT KUB and it showed a paravertebral collection which was drained and grew Salmonella Type D organisms as well confirming dissemination and seeding. Over the course of her hospital stay, she developed cervical lymphadenopathy which was not clinically significant on the day of admission. The largest node was excised and routine bacterial culture was negative, however MOTT was grown and identified as MAC. She completed a course of IV Ceftriaxone covering Salmonellosis and was started on anti-MOTT treatment and discharged home few weeks after admission. Six months post discharge she presented to our hospital with fever, productive cough and recurrent abdominal pain. Repeated workup confirmed the growth of MAC again from sputum, blood and lymph node biopsy with the same sensitivity pattern as the initial strain. A new intensive cycle of anti-MAC using Moxifloxacin, Ethambutol, Rifampicin and Clarithromycin was initiated and she showed marked clinical improvement with symptomatic improvement and regression of cervical lymphadenopathy. The autoantibodies against IFN- ?? were negative. The patient completed Anti-mycobacterial therapy for 24 months with regular follow up in the clinic till end of December 2016.

Conclusions: Young healthy adults with relative good immunity can develop disseminated infections secondary to ubiquitous and common environmental organisms due to abnormal IFN- ?? and IL-12 signaling pathways. The managing clinician should maintain a high index of suspicion when managing non-AIDS patients presenting with disseminated MAC especially with concurrent invasive gram-negative infections and should work up the patients for underlying rare immune defects.

P 83

Mortality in patients with Mycobacterium avium complex lung disease: a review of published literature

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Background: Nontuberculous mycobacteria (NTM) are ubiquitous environmental bacteria and the prevalence in human clinical samples is increasing worldwide. Mycobacterium avium Complex (MAC) has been reported to be the most common causative agent in NTM lung disease worldwide. Treatment outcomes in NTM lung disease are sparsely reported and as of today only one systematic literature review on quality of life, co-morbidities and mortality, and one meta-analysis on treatment success rate have been published. However, no review of long-term mortality specifically in patients with MAC lung disease has been published yet.

Aim: The objective of the analysis was to collect available data from the published literature on 5-year all-cause mortality in patients with MAC lung disease and to explore study characteristics that may have contributed to variability in all-cause mortality reports.

Results: We have identified 13 published studies reporting 5-year mortality in patients with MAC lung disease. Ten studies were retrospective and three were prospective, with sample sizes from 9 to 782 patients with MAC lung disease. 5-year mortality rates ranged between 10% and 66%, and 10 of the 13 studies reported a rate exceeding 25%. The Q-statistics (Q=172, degrees of freedom (df)=12) suggest substantial deviations of study-specific mortality from an aggregate mortality estimate. The I^2-statistic (I^2=93%) indicates that 93% of the observed variability in mortality rates was likely due to true heterogeneity in mortality rates among the studies. Lower mortality rates were reported by studies in patients with predominantly nodular disease, whereas higher rates by studies in patients with predominantly cavitary disease or in case of macrolides resistance.

Conclusions: Risk of all-cause mortality in patients with MAC lung disease varies across studies. Most of the studies document a 5-year mortality rate greater than 25%, indicating a substantial health threat to people with the disease.

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Non-tuberculous Mycobacteria: one year of isolations in metropolitan area of Milan

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Introduction: Non-tuberculous Mycobacteria (NTM) are widely distributed in the environment; they are frequently isolated and are responsible of symptomatic disease. The most common disease is a lung infection, but lymphatic and skin/soft tissue ones are also important.

It's difficult to eradicate NTM and a prolonged therapy with drugs combination is required.

The aim of our work was an epidemiological evaluation of NTM isolated in our lab in 2016.
Material and methods: In 2016 we have isolated strains of NTM considered pathogenic following American Thoracic Society Criteria. The identification was performed using GenoType Mycobacterium CM/AS (Hain LifeScience) or 16S sequencing. The sensitivity was tested using Sensititre™ SLOMYCOI or RAPMYCOI (Termoscientific) following CLSI guidelines.
Results: 158 patients of our center were affected by an NTM disease.
Females were 95 and males were 63; the mean age was 59 (median 66). The mean age for pulmonary disease was 63,1, whereas for lymphnodal disease was 2.3 years.
In lung diseases we isolated: 41,4% *M. intracellulare*, 35,7% *M. avium*, 7,8% *M. abscessus*, 4,3% *M. xenopi*, 4,3% other rapid growing NTM, 6,4% other slow growing NTM. In lymphnodal disease 75% of isolated strains were *M. avium*.
We performed 18 sensitivity test for Rapid NTM and 132 for Slow NTM.
Conclusions: *Mycobacterium avium* complex (MAC) is the most common pathogen isolated in lung diseases (77,9%) followed by *M. abscessus* and *M. xenopy*; these data reproduce the NTM European distribution on respiratory samples. Our distribution of MAC in lung diseases is different from the global one, because the percentage of *M. intracellulare* is higher relative to *M. avium*.
Regarding Sensitivity tests for all NTM, we haven't observed any significant difference for M.I.C. of antibiotics relative to the expected.

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Effects of the depletion of essential early and late division proteins in Mycobacterium smegmatis

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Mycobacterial growth and division are characterised by their asymmetry that may produce heterogeneous populations with variable cell morphology and different cell-to-cell antibiotic susceptibility. The heterogeneity is likely imposed by the particular properties of the mycobacterial cell division and peptidoglycan synthesis machineries.
For cell division, bacteria assemble in a sequential way a complex of several essential proteins that is called the divisome. We distinguish between early division proteins, which form the FtsZ containing proto-ring, and late ones, which connect the proto-ring to the peptidoglycan synthesis machinery. Mycobacteria have a reduced set of divisome components and recently the essential SepF protein was shown to form part of the early assembling proto-ring.
To further characterise the mycobacterial divisome, conditional *Mycobacterium smegmatis* mutants in early and late divisome genes were obtained. The effects of the depletion of their products on growth and division were then investigated. We found substantial differences in the kinetics of depletion of SepF and the periplasmic connector, with the latter requiring several doubling times of repression to produce a defect in cell division. This might have consequences for the suitability of these proteins as targets for antibiotics that act on the level of the division machinery.

P 125

Plasmid carriage in Mycobacterium chimaera

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Several recent studies have identified water tanks of Heater–cooler units (HCUs) as potential sources of *Mycobacterium chimaera*, causing a number of surgical site infections. Recently, we identified *M. chimaera* in HCUs from all five thoracic surgery departments in Denmark and subjected a total of seven *M. chimaera* isolates to whole-genome sequencing. From each sequenced isolate, we were able to recover circular genomes of 4-6 extrachromosomal elements (plasmids) ranging between 13-157 kbp in size. By analyzing the plasmid profiles of our isolates and other *M. chimaera* isolates retrieved from the European Nucleotide Archive (ENA) we were able to establish that 97% of sequenced isolates contained one or more plasmids identical to plasmids present in Danish isolates. Remarkably, all *M. chimaera* isolates associated with the Sorin Stöckert 3T HCUs carried a plasmid not seen in any other isolates. Thus, we have established that *M. chimaera* can carry distinct plasmid profiles that can be tied to specific isolation sources.

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Frequency of isolation of Mycobacterium chimaera in Croatia, 2013-2016

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Growing evidence of invasive cardiovascular infections caused by *Mycobacterium* (*M.*) *chimaera*, member of *M. avium* complex, raised the awareness of its clinical significance. Contaminated heater-cooler units used in cardiac surgery were pointed out as a potential source of infection. As *M. chimaera* and *M. intracellulare* are genetically very close, adequate techniques should be used to accurately identify the species. Using commercial GenoType NTM-DR assay (Hain Lifescience, Germany), it is possible to distinguish these species.
In 2016, *M. chimaera* was detected in four out of six water samples from heater-cooler units inside operating theatres from two different hospitals in Croatia. After these findings, we performed retrospective analysis of *M. intracellulare* strains isolated from patients' samples from 2013 to 2016. Majority of strains were isolated from respiratory samples and several patients had more than one *M. intracellulare* isolate. A total of 28 *M. intracellulare* strains was analysed using GenoType NTM-DR. We found that *M. chimaera* represents 25% (n=7) of the isolates previously identified as *M. intracellulare*. None of the patients with *M. chimaera* isolate had more than one positive culture. The clinical relevance of *M. chimaera* isolates is still to be determined.

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Atypical Mycobacteria identified in recent years in Córdoba (Spain)

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For a long time, isolates of atypical Mycobacteria have been an important chapter in infectious pathology. The development of modern microbiological diagnostic systems has allowed a better isolation and identification of these species, especially those of difficult growth in conventional solid media and difficult to typify by classic biochemical methods. New liquid culture media, genetic technology, chromatographic systems of mycolic acid studies, mass spectrophotometry etc have contributed significantly to a better diagnosis of these species. The PCR technique Genotype CM / AS, allows the identification of the species of Mycobacteria that are most frequently isolated in man, many of them with clinical significance.
Objective: To present the different species of atypical Mycobacteria isolated in our Center in the period 2005-2015.
Material and methods: In this period, 466 atypical mycobacterial cultures have been identified, either from Lowenstein-Jensen solid media or liquid media from MGIT. Identification has been made using the Genotype CM and Genotype AS, according to the established protocol.
Results: The 466 cultures identified as atypical Mycobacteria, belonged to 24 different species. The most frequently identified was *Mycobacterium intracellulare*, 102 cultures (21.88%) followed by *Mycobacterium fortuitum* with 89 cultures (19.09%), *Mycobacterium chelonae* and *Mycobacterium gordonae* with 37 cultures (7.93%), Mycobacterium abscessus, 29 cultures (6.22%), and in a smaller proportion, *Mycobacterium kansasii* and *Mycobacterium lentiflavum* with 15 cultures , *Mycobacterium marinum* 13 (2.78%), *Mycobacterium mucogeni*cum 8 (1.71%), *Mycobacterium peregrinum* and *Mycobacterium simiae,* 7 (1.5%), *Mycobacterium scrofulaceum*, 6 (1.28%), *Mycobacterium spp.,* Of the species identified as *Mycobacterium interjectum, M. shimoidei, M. gadium* and *M. diernhoferi*, only one crop of each was identified in this period. Throughout these years of study, the year with the most identified crops was 2011, followed by 2012,2008 and 2010. In 2010 and 2011, the most identified species was *M. fortuitum*; in 2012, *M. intracellulare*, and in 2008, *M. avium.*
Conclusion: Given that the isolation of atypical mycobacteria continue to increase over the years, and that many of them are associated with cases of immunosuppression, and that these species are easily identified by genetic systems, mass spectrometry etc., it maintains an open field of study mainly oriented to the identification of species that would escape this genetic system.

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The role of mycobacterial infections in keratitis cases in Cukurova Region, Turkey

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Recently, the role of mycobacteria, especially nontuberculous mycobacteria (NTM), has become increasingly important in microbial keratitis whose differential diagnosis is challenging. Because of difficulties in routine mycobacterial identification, mycobacterial keratitis is commonly confused with fungal, herpetic and amoebic keratitis, and the worldwide incidence of this infection is low, thereby leads to the failure of treatment strategies overlooking mycobacteria and the emergence of persistent or recurrent infections.
Therefore, we aimed to investigate the presence and the role of mycobacterial infections in keratitis cases in Cukurova Region, Turkey.
Corneal scraping samples collected from 50 patients who are diagnosed as keratitis in a major regional hospital between December 2016 and March 2017, were inoculated on Löwenstein-Jensen (LJ) and MGIT 960 liquid system, direct preparations stained with EZN method were evaluated microscopically. The presence of mycobacteria was investigated by using PCR assay with specific primers targeting *hsp65* gene region subsequent to DNA extraction directly from the corneal scrapings by using acid washed mini-glass beads in Mickle cell disruptor.
According to the results of our study, only one isolate was identified as *M. fortuitum* with both culture and PCR methods.
As a result of our research which will be the first study examining the presence and the role of mycobacterial infections in keratitis cases in our country by using phenotypical and molecular methods, the expected achievement of early and accurate making diagnosis for overlooked mycobacterial keratitis will enable us to plan the treatment strategies rationally by rapid detection of drug resistance. Our study is ongoing.
Key Words: DNA sequence analysis, *hsp65,* keratitis, mycobacterial infection, PCR, nontuberculous mycobacteria (NTM)

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Antimicrobial Susceptibility Results of Clinically Significant Rapidly Growing Mycobacteria

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Aim: Rapidly growing mycobacteria (RGM) are commonly found in the environment. RGM infections can cause several diseases with significant morbidity and mortality. Treatment of RGM pulmonary infection is a difficult process for both the patient and the clinician. It was to detect antimicrobial susceptibility of rapidly growing mycobacteria (RGM) isolated from various clinically suspected cases of pulmonary and extrapulmonary tuberculosis.
Method: In this study, it was used a total of 96 strains (48 *M. fortuitum*, 31 *M. abscessus*, and 17 *M. chelonae*) from the most isolated RGM in our laboratory. The specimens were collected from various regions in Turkey between June 2015 and December 2016. RGM were identified by GenoType Mycobacterium CM/AS assay (Hain Lifescience). Antimicrobial susceptibility for RGM was tested by Sensititre RAPMYCO panel (Trek DiagnosticSystems), for the Clinical and Laboratory Standards Institute (CLSI) recommends broth microdilution method to determine Minimal Inhibition Concentration (MIC) in susceptibility testing of RGM. The 96- well plates were evaluated after 5 days of incubation. The MIC results of all strains to amikacin, cefoxitin, ciprofloxacin, clarithromycin, doxycycline, imipenem, linezolid, sulfamethoxazole, tobramycin and moxifloxacin were interpreted.
Results: Almost all of *M. abscessus* (97%), *M. fortuitum* (92%) and *M. chelonae* (94%) strains were susceptible to amikacin, whereas *M. abscessus* (90%) and *M. fortuitum* (100%) were resistant to tobramycin. It was found that almost all *M. chelonae* strains were susceptible to clarithromycin (94%) and linezolid (76%), while *M. chelonae* was resistant to doxycycline (71%), imipenem (71%), sulfamethoxazole (64%) and moxifloxacin (53%). *M. fortuitum* was susceptible to ciprofloxacin (85%), clarithromycin (56%), linezolid (85%), moxifloxacin (92%) and sulfamethoxazole (100%). *M. abscessus* was susceptible to ciprofloxacin (81%), clarithromycin (77%), linezolid (97%), moxifloxacin (77%) and sulfamethoxazole (68%), while *M. abscessus* was resistant to doxycycline (65%) and imipenem (61%).
Conclusion: RGM should be identified and drug susceptibility tests of every clinically significant isolate should be done before the effective treatment of infections.
Key words: Nontuberculous Mycobacteria, *M. abscessus*, *M. fortuitum*, *M. chelonae*, Antimicrobial susceptibility

P 46

Point source introduction of Mycobacterium bovis at the wildlife-livestock interface can lead to clonal expansion of the disease in a single ecosystem

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Mycobacterium bovis infects multiple wildlife species and domesticated cattle across South Africa, and negatively impacts on livestock trade and movement of wildlife for conservation purposes. *M. bovis* infection was first reported in the Kruger National Park (KNP) in South Africa during the 1990s, and has since spread to infect numerous animal host species throughout the park and across South Africa. Whole genome sequencing data of 17 *M. bovis* isolates were analyzed to investigate the genomic diversity among *M. bovis* isolates causing disease in different animal host species from various locations in South Africa. *M. bovis* strains analyzed in this study are geographic rather than host species-specific. The clonal expansion of *M. bovis* in the KNP highlights the effect of an introduction of a transmissible infectious disease leading to a rising epidemic in wildlife, and emphasizes the importance of disease control and movement restriction of species that serve as disease reservoirs. In conclusion, the point source introduction of a single *M. bovis* strain type in the KNP ecosystem lead to an *M. bovis* outbreak in this area that affects various host species and poses an infection risk in neighboring rural communities where HIV prevalence is high.

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First report of Mycobacterium africanum in buffaloes and cattle in Marajo Island, Brazilian Amazon

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Background: *Mycobacterium tuberculosis* complex (MTBC) is a versatile group of species, capable of infecting a wide variety of animals. In livestock animals, the etiology of the disease had been traditionally imputed to *M. bovis,* however previous studies using molecular approaches already strongly suggest its low prevalence in lesions of animals in Brazilian Amazon.
Materials and methods: Between 2014 and 2016 were examined 66 animals (42 cattle and 24 buffaloes) from Marajo Island, of which were collected biopsies from lesions with clinical features suggestive of tuberculosis obtained from sterile anatomical sites. The samples were inoculated into Lowenstein-Jensen medium after decontamination by the N-acetyl-L-cysteine-sodium hydroxide procedure and incubated at 35 to 37°C in the absence of light for at least six weeks or until colonies appeared. Isolates of the MTBC were distinguished from nontuberculous mycobacteria (NTM) by the colony morphology aspect and molecular analysis. Genomic DNA was extracted of all mycobacteria isolates and analysis of the sequences of 16S rRNA, *hsp65,* and Exact Tanden Repeat D (ETR-D) were used to identify MTBC. The generated sequences were compared with references sequences deposited in GenBank.
Results: A total of 34 mycobacteria were

isolated (18 MTBC and 16 NTM), belonging to 18 cattle and 16 buffaloes. Among MTBC, all isolates (belonging to 9 cattle and 9 buffaloes) presented sequences with nucleotide signature of *M. africanum* species by ETR-D.
Conclusion: The findings of this study suggests that *M. africanum* may be the main species from the MTBC affecting livestock animals in Marajo Island, Brazilian Amazon. However, further epidemiological investigations are required in order to better describe the species prevalence in the region.

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Detection of Mycobacterium bovis in bovine lung and liver tissues bought in butcher shops from the Grater Buenos Aires, Argentina

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Bovine tuberculosis (BT) is caused by *Mycobacterium bovis* (*M. bovis*). In Argentina, BT prevalence is 0.2%, based on the detection of tuberculous lesions during slaughterhouse inspections. Our country has approved a national BT program for the control and eradication of the disease.
However, the *M. bovis* infection is still detected in low rate in humans, other animals and pets. *M. bovis* infections in cats may be explained by the popular custom of feeding them with raw beef lungs or livers.
The hypothesis of this work was that, due to the characteristics of the chronic infection, microscopic tuberculous lesions in bovine organs might potentially pass undetected during official inspections in slaughterhouses, allowing contaminated tissue to be commercialized.
Two hundred lungs and 6 livers sold in 6 butcher shops and 1 slaughterhouse of the Greater of Buenos Aires were sampled. Sixteen isolates were obtained in Stonebrink medium, and Ziehl Neelsen staining and IS-*6110* PCR assay have been performed. Out of these, 5 isolates were identified as *M. bovis* by spoligotyping.
One isolate was obtained from a lung purchased in a slaughterhouse from the western region of the Greater Buenos Aires, another one from a lung bought in a butcher shop from the northern region and the other 3, obtained from 2 lungs and 1 liver, from the same butcher shop in the southern region.
Lesions compatible with tuberculosis were observed in the sample from the liver, which could only be evidenced after the liver was sliced in the laboratory. Additionally, 11 isolates were negative to IS-*6110* PCR assay, and they were thus identified as Mycobacteria other than tuberculosis by sequencing of the 16S RNA ribosomal gene. The *M. bovis* genotyping was performed using spoligotyping and MIRU-VNTR. Three genotypes were detected by spoligotyping: SB0120, SB0130 and SB0140 whereas by MIRU-VNTR 3 patterns were detected. All the spoligotypes obtained in this study are often identified in bovines from Argentina.
The results presented in this study confirm the presence of viable *M. bovis* in commercialized organs, which proves escapes from the slaughterhouse inspection. The manipulation of raw giblets or their use to feed pets is a risk factor because it could contain viable mycobacteria that may cause the disease. Due to the importance of this disease and the risk of zoonotic and epizootic transmission, we suggest strengthening the controls during the inspection in slaughterhouses.

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MALDI-TOF and 16S rDNA sequencing for identification of nontuberculous mycobacteria in a veterinary laboratory – a diagnostic challenge

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In order to facilitate and accelerate the identification of nontuberculous mycobacteria (NTM), a small-scale comparative study based on mass spectrometry and sequencing was carried out. After selection on the basis of Ziehl-Neelsen staining and colony morphology, a total of 32 mycobacterial strains isolated from internal organs and lymph nodes of wild boars were subjected to identification with MALDI-TOF and 16S rDNA sequencing.
The selected mycobacteria originated from 23 animals. Most frequently, one isolate per animal was obtained. However, in three cases two isolates per animal were obtained, in one case three isolates per animal and in another five isolates per animal. All isolates from the same animal were of identical mycobacterial species, with the exception of one animal where

mycobacterial species differed according to the sample type. Eight animals were positive for *M. nonchromogenicum*, six for *Mycobacterium* spp., three for *M. peregrinum* (one of these was also positive for *M. nonchromogenicum*) and two for *M. avium.*, in total 18 animals. Three animals were positive for *M. septicum*, *M. arupense* or *M. thermoresistibile*, respectively. Mycobacteria could not be identified in two of the inspected animals.
MALDI-TOF enabled reliable species identification in 47% (15/32) of isolates: *M. nonchromogenicum* (n=6), *M. avium* (n=5), *M. peregrinum* (n=1), *M. septicum* (n=1), *M. arupense* (n=1) and *M. thermoresistibile* (n=1). Sequencing provided unambiguous results to the species level in 53% (17/32) of isolates: *M. nonchromogenicum* (n=10), *M. avium* (n=6) and *M. arupense* (n=1). Combination of both methods resulted in species identification of 72% (23/32) of isolates: *M. nonchromogenicum* (n=10), *M. avium* (n=6), *M. peregrinum* (n=4), *M. septicum* (n=1), *M. arupense* (n=1) and *M. thermoresistibile* (n=1). Identification to the species – and also to the genus level – failed for 10, 3 or 2 isolates when using MALDI-TOF, sequencing or both methods, respectively. Other isolates could be identified to the genus level only.
Results show that 16S rDNA sequencing gives more unambiguous identification of NTM, since it enabled identification to the species level for more isolates; in addition, identification failed in fewer cases. The combination of MALDI-TOF and sequencing resulted in the highest number of species identifications as the identity of only two isolates could not be assessed.

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Tuberculosis in congolese children infected with HIV with severe complicated acute malnutrition, Tshikaji Bon Berger Hospital -DRCongo

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Context: The considerable increase in lung infections due to tuberculous bacilli other than tuberculosis complexes poses a real public health problem. Their strong responsibility in various human pathologies such as tuberculosis-like lung infections, extrapulmonary infections or those disseminated especially in immunocompromised patients deserves special attention.
Objective: Identify the Mycobacterium tuberculosis complex and other isolates using the DNA-STRIP molecular assay.
Methods: We carried out a study of 208 samples collected between January 2011 and June 2012. The samples received were decontaminated by the N acetyl cysteine sodium method and then seeded on automated liquid media and lowenstein Jensen. DNA extraction on the positive cultures obtained was performed by the chloroform method prior to identification with the mycobacterium CM genotype kit and by Maldi-TOF mass spectrometry.
Results: Our study population comprised 59.7% males and 39.5% females for an age range of 11 months to 87 years. New cases accounted for 93%, reprocessing (4%), relapse (1%), and mostly from services such as internal medicine, pneumology and pediatrics. Positive cultures for atypical mycobacteria accounted for 25.4% of the total crop versus 74.5% for mycobacteria of the tuberculosis complex. M.fortuitum was the most isolated atypical mycobacterial species (18.75%), followed by intracellular M. (12.5%). M. gordonae (5.6 per cent); M. abscessus (3.7%); M. intracellular (3.7%) and M. malmoense (1.8%). However, 39% of the atypical mycobacteria isolated could not be identified by the kit Genotype Mycobacterium CM used and by mass spectrometry.
Conclusion: The diversity of strains of Mycobacteria isolated from hospitalized patients build a real warning sign of the involvement of these bacilli in certain pulmonary conditions. Their high resistance to antibiotics, combined with the complexity of their clinical presentation, hinders their treatment and consequently the optimal management of patients infected with these bacilli.

New diagnostics, new diagnostic algorithms, new treatment regimens

P 5

Optimization of culture media Lowenstein-Jensen and Stonebrink to improve the growth of Mycobacterium spp.

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The genus Mycobacterium includes an important group of pathogens that causes chronic diseases such as tuberculosis, leprosy and Buruli ulcer, affecting animals and humans. In vitro culture is considered as gold standard test, although its main disadvantage is the long period of time to observe colonies on the medium. The aim of this study was to optimate the main culture media used in the diagnosis of mycobacteriosis, Lowenstein-Jensen (LW) and Stonebrink (ST). Three supplements were evaluated, i.e., horse serum, ferric ammonium citrate and tween 80, applied at different concentrations on the growth of five inbred strains: M. tuberculosis, M. bovis, M. bovis BCG variety, M. kansasii and M. ulcerans. In total, 810 tubes with LW and ST media were inoculated and cultured comprised in 27 treatments; all samples showed growth for all strains in 6 to 8 days. Following a methodology of response surface in the statistical analysis, it was possible to identify the best formulation in stimulating a better mycobacterial growth. The results showed that M. tuberculosis complex strains had a better growth in ST media supplemented with 0.3g /L CFA, 0.05% Tween 80 and 7.5-10% serum, for M. kansasii strains in ST media supplemented with 0.3g / L CFA, 0.2 % tween 80 and 10% serum, and for M. ulcerans strains in ST media supplemented with 0.04g / L CFA, 0.2% tween 80, and 5% serum. The use of supplements added in LW and ST media increased significantly the growth of M. tuberculosis complex strains, the importance of adding iron to stimulate the development of strains was proved, except in M. bovis BCG variety; in addition, the use of tween 80 for atypical mycobacteria and serum for both mycobacterial groups, was demonstrated.

P 6

Global transcriptional analyses of Mycobacterium tuberculosis using old and new drugs

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Background: Current TB drug resistance research is mainly focused on the genomic level, using WGS to search for resistance-causing mutations. However, transcriptional analyses can be a powerful tool as well. Analyses on susceptible strains provide information on the response to the stress a drug is causing, showing which mechanisms are activated to counter the effect of the drug. This might lead to new strategies for the development of new/complimentary drugs. On the other hand, this response gives a view on the working mechanism of the drug. Moreover, comparing the response of sensitive and resistant strains can yield additional information, like the activation of specific efflux pumps.
However, due to the high cost of RNAseq analyses, genome wide transcriptional analyses of drug influences on *M. tuberculosis* remain rather limited. Here we present a global study of the response of two pansensitive *M. tuberculosis* strains to eight TB-drugs. By using the approach called RNAtag-Seq, multiple samples were combined in one run, reducing time and cost.
Materials/Methods: For each drug (isoniazid, rifampicin, ethambutol, capreomycin, amikacin, linezolid, moxifloxacin and bedaquilin), twice the critical concentration was added to the cultures of two pansensitive *M. tuberculosis* strains. Samples were taken 0h, 2h, 6h and 24h after the administration of the drug. RNA was extracted combining bead-beating in TRIzol and the Direct-Zol purification kit. After quality control, the extracts were fragmented, barcoded and pooled, cDNA libraries were constructed and sequenced using the HiSeq technology (Illumina®). Reads were mapped to the reference genome and

normalized read counts were calculated per gene.
Results: For each drug a specific transcriptional response was mapped, revealing lists of up- and down-regulated genes. As an example and in accordance with previous studies, the highest induced genes in the presence of isoniazid belong to a cluster of genes that encodes components of the FAS-II (fatty acid synthase II) complex, which is targeted by isoniazid. On the other hand, a remarkable down-regulation of the NADH-dehydrogenase (ndh) cluster genes was noted. This can be assigned to the dependence of isoniazid target inhA on NADH. Lowering the ndh-activity is also seen in resistant strains.
Conclusions: By using RNAtag-seq, a global study of the transcriptional response of *M. tuberculosis* to several drugs could be made. These analyses can reveal the working mechanisms of existing and new drugs and provide new insights on the mechanism of resistance of strains.

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The identification of non-tuberculous mycobacteria species in russian tuberculosis patients using MALDI-TOF mass spectrometry

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Identification of non-tuberculosis Mycobacteria (NTM) species in respiratory patients remains an issue especially in settings where availability of molecular-based techniques is limited. The aim of the study was to evaluate the feasibility of NTM species identification using MALDI-TOF mass spectrometry.
Materials and methods. MALDI Biotyper (Bruker Daltonics, MA, USA) system was used for the identification of 78 strains isolated from TB patients in Samara, Russia in 2015-2016. Results were compared with those of molecular identification using GenoType® CM and AS kits (Hain Lifescience®, Nehren, Germany).
Results. Sixteen M. kansasii (20.5%), 11 M. avium (14.1%), 11 M. fortuitum (14.1%), 9 M. gordonae (11.5%), 3 strains (3.8%) of M. peregrinum, M. szulgai, and M. chimera intracellulare group; 2 strains (2.6%) of M. abscessus, M. septicum, M. paragordonae, and M. senegalence, and 1 strain (1.3%) of M. chelonae, M. frederiksbergense, M. monacense, and M. lentiflavum were identified using MALDI-TOF mass spectrometry. Using this method, 15 NTM species were identified while only 9 species were identified using DNA hybridization approach. Identification results of two methods was concordant in only 45 (57.7%) strains, while in 16 strains (20.5%) results were discordant. Seventeen bacterial strains (21.8%) isolated from TB patients but not identified by DNA hybridization method were identified by MALDI-TOF mass spectrometry. They included 7 slow-growing strains (9.0%) not belonging to Mycobacteria genus – Gordonia rubriperticta, Nocardia forcinica, Tsukumurella spp., and Rhodotorula mucilaginosa.
Conclusion. Performance of existing molecular methods for species identification can be limited as it is shown in our study, especially in cases of non-Mycobacterial respiratory infection. Implementation of new methods, including MALDI-TOF mass spectrometry, makes species identification more sensitive and allows to identify wider range of NTM species as well as other bacteria species similar to Mycobacteria in terms of morphological and physiological characteristics.

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Resuscitation of latent M. tuberculosis?

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Context: *Mycobacterium tuberculosis* has the ability to latently reside in humans without causing clinical symptoms, a state known as latent tuberculosis infection (LTBI). Dormant latent bacteria remain undetected by conventional culture methods. Therefore, these "invisible" dormant bacteria represent a diagnostic target, which if successfully detected may improve tuberculosis control hindered by LTBI. Our previous studies identified that high concentration of vitamin K led to explosive resuscitation of the dormant blood microbiota.
Aim of our study was to test a novel approach

based on resuscitation of dormant *M. tuberculosis* in blood samples.

Methods: Blood was collected using Vacutainer K3E 5 ml tubes from 28 healthy LTBI negative volunteers and two volunteers with confirmed LTBI by Mantoux tuberculin skin test and Interferon-gamma release assay (IGRA) and no previous treatment for TB. Blood cultivation 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. Isolated blood microbiota were confirmed by Gram staining, TEM and 16S rRNA genes PCR analysis. Eight commercial kits were tested for DNA isolation of the cultured blood microbiota. For DNA analysis we applied 16S rRNA genes, 18S rRNA genes and ITS2 targeted sequencing on Illumina MiSeq. The obtained sequences were clustered (≥97% identity) in Operational Taxonomic Units (OTUs). OTUs were analyzed for sequence similarities against reference sequence databases; Greengenes for 16S rDNA, Silva for 18S rDNA and UNITE for ITS2.

Results: Isolation of blood microbiota was equally effective from lysed or whole blood, as confirmed by Gram staining and TEM. TEM images demonstrated well defined cell structures. Most promising result for DNA isolation regarding purity and quantity was obtained with the RIBO-prep kit optimized with steps of freeze-thawing and DNA repurification with Chelex 100. On the bases of the 16S rRNA genes, 18S rRNA genes and ITS2 sequencing results we identified OTUs similarity with 25 bacterial genera belonging to 4 phyla and 10 fungi genera (predominantly *Alternaria, Epicoccum, Monographella, Malassezia, Filobasidium* and others) belonging to 2 phyla. Optimization of the conditions for molecular confirmation of resuscitated LTBI dormant bacteria in blood samples is ongoing.

Conclusions: Resuscitation of the blood *M. tuberculosis* of LTBI is highly challenging. Preliminary results suggest that using vitamin K to reanimate the dormant bacteria may enable quantitative detection of the dormant *M. tuberculosis* population in patients with LTBI and holds promise for becoming a predictive tool for LTBI dynamics. We hypothesize that the amount of dormant MTB bacteria in LTB patients is related to the level of risk for TB activation.

Acknowledgements: This study was supported by Grant ДН-01-4/16.12.2016 from the National Science Fund of Bulgaria.

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The first evaluation of the diagnostic performance of the Fluorotype MTBDR assay for the detection of Mycobacterium tuberculosis and resistance to rifampicin and isoniazid

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There has been a paradigm shift in the way that drug susceptibility testing is done routinely. Since 2008 two assays have been approved by the WHO. In South Africa Xpert MTB/RIF has been universally implemented as the primary screening tool, however these results need to be confirmed by a secondary assay, namely the Genotype MTBDRplus. This confirmatory test requires extensive laboratory infrastructure to prevent laboratory cross contamination, has multiple steps and only provides limited information on nucleotide variants conferring resistance.

The objective was to evaluate the diagnostic performance of the Fluorotype MTBDR (FTMTBDR) assay using smear positive and smear negative sputum specimens as well as cultured isolates.

Sputum specimens and correlating cultivated samples were retrospectively collected from the NHLS, Green Point, South Africa. A total number of 555 samples were collected for the study and tested with the FTMTBDR assay, using FluoroLyse kit as DNA extraction method and the FluoroCycler96 for amplification and detection. This included 244 smear positive/culture positive sputum specimens, 99 smear negative/culture positive sputum specimens, 105 smear negative/culture negative sputum specimens, and 107 cultured isolates. MGIT culture and Genotype MTBDRplus VER2.0 (GTMTBDR) results from the cultured isolates were used as method of comparison. Discrepancies were resolved by sequencing and the GTMTBDR assay from sputum specimens.

The sensitivity for the detection of *M. tuberculosis* using the FTMTBDR assay in smear positive sputum specimens, smear negative sputum specimens and cultured isolates was 97.9%, 91.8% and 100% respectively. The sensitivity and specificity for the detection of rifampicin and isoniazid resistance in smear positive sputum specimens was 99.2% and 100%, and 100% and 99.1% respectively. The sensitivity and

specificity for the detection of rifampicin and isoniazid resistance in smear negative sputum specimens was 100% and 97.3%, and 100% and 97.8% respectively. When compared to sequencing and the GTMTBDR results, the FTMTBDR assay showed a 97.9%, 97.0% and 97.2% accuracy for the correct identification of mutations in *rpoB*, *katG* and the *inhA* promoter region respectively.
The FTMTBDR assay was highly concordant to that of the GTMTBDR assay without the subjectivity of visually interpreting the hybridization patterns. Besides the discrimination of rifampicin and/or isoniazid resistance, the software also allows for the reliable identification of most significant associated mutations found in *rpoB, katG* and the promoter region of *inhA*.

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Genotypic and phenotypic correlation of delamanid susceptibility in delamanid naive Mycoacterium tuberculosis clinical isolates

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Drug resistance continues to threaten TB control in South Africa despite the implementation of rapid diagnostics and standardized multidrug-resistant (MDR) regimens. To address the global MDR epidemic the WHO has issued new guidelines for the treatment of rifampicin resistant or MDR-TB. These guidelines includes the use of bedaquilone, delamanid, clofazamine, linezolid to ensure that MDR-TB patients are treated with a regimen that consists of a minimum of five effective anti-TB antibiotics. However routine drugs susceptibility testing is only done for isoniazid, rifampicin, ofloxacin and amikacin. This implies that patients are being treated with anti-TB drugs without knowing whether the infecting strains are resistant. Currently, the level of spontaneous base-line resistance to base-line resistance to delamanid remains unknown in South Africa despite its planned use for the treatment of MDR-TB. Previous studies have demonstrated an association between sequence variants in the genes responsible for the activation of delamanid (*fbiA*, *fbiB*, *fbiC*, *fgd* and *Rv3547*) and phenotypic resistance. In collaboration with Otsuka, our group has also previously shown an association with a genomic insertion (>10kbps) in *Rv3547* (resulting in loss of function) and delamanid resistance. In an attempt to determine the base-line level of delamanid resistance we have reviewed the genome sequences of over 2000 drug susceptible and resistant *Mycobacterium tuberculosis* delamanid naïve isolates. From this sample set we identified 83 isolates showing variants (single nucleotide polymorphisms and short insertions and deletions) in the five genes, from which 45 showed variants that have previously been shown to be associated with delamanid susceptibility. The remaining variants identified in this sample set are however novel and have not been previously described. This study aim to determine the correlation between variants found in *fbiA*, *fbiB*, *fbiC*, *fgd* and *Rv3547* and phenotypic resistance to delamanid in *M. tuberculosis* clinical isolates collected from delamanid naïve patients. Delamanid drug susceptibility testing will be done on the complete set of 83 isolates using the BACTEC MGIT960 system at the 0.04 µg/ml and 0.125 µg/ml. Identification of molecular markers associated with delamanid resistance will guide the development of novel genotypic diagnostic assays. The identification of spontaneous resistance in delamanid naïve isolates highlights the need for phenotypic drug susceptibility testing before initiation of treatment, in order to prevent the selection and further transmission of delamanid resistant strains.

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The Mycobacterium tuberculosis P-type ATPase plasma membrane transporter CtpF as potencial drug target

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The emergence of multi-drug resistant (MDR) and extensively drug resistant (XDR) *Mycobacterium tuberculosis* strains has driven the finding of more effective anti-TB drugs. Currently, the plasma membrane proteins has been considered as key drug targets due to their biological implication and high accessibility to the active compounds. Mycobacterial P-type ATPases could be interesting anti-TB targets due to their role in ion homeostasis across the plasma membrane and mycobacterial virulence. It was previously observed that CtpF, a putative Ca^{2+}-ATPase of *M. tuberculosis*, is activated under hypoxia and infection conditions; therefore, this pump is a potential drug target.
CtpF has been shown homologous with the sarco (endo) plasmic reticulum Ca^{2+}-ATPases (SERCA). A structure-based sequence alignment

between CtpF and SERCA crystal structures in the presence of inhibitor cyclopiazonic acid (CPA) reveals that the structurally important residues for CPA binding are conserved. Molecular docking showed that the binding mode is similar to those in SERCA crystal structures PDBs (2O9J, 3FPB4, 4YCL). In conclusion, we consider CPA binding pocket as a suitable starting point for the rational design of anti-TB compounds.

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Mycobacterial genomic DNA from used Xpert MTB/RIF cartridges can be used for second-line genotypic drug susceptibility testing and spoligotyping

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Xpert MTB/RIF is a widely used automated PCR test for TB that also detect rifampicin resistance. If patients require further drug susceptibility testing, a second specimen is collected. This can increase diagnostic delay and pre-treatment loss to follow-up. We examined whether DNA from used Xpert cartridges was useful for genotypic drug-susceptibility testing (which may obviate the need for collection of a second specimen), spoligotyping (which may be useful to programmes looking to incorporate molecular epidemiology), and sequencing (which may be useful for genotypic drug susceptibility testing).
TB-positive Xpert cartridges done on triplicate dilution series (0-10^6 CFU/ml) of previously-described drug-susceptible (DS), multi-drug resistant (MDR) and extensively-drug resistant (XDR) strains had ~15 µl extracted. This extract was used for MTBDR*plus* and MTBDR*sl* (two World Health Organization-approved drug susceptibility tests), and *gyrA* Sanger sequencing (~5 µl each; sequencing not done on MDR-TB strain). MTBDR*plus* and MTBDR*sl* were also done on extract from Xpert rifampicin-susceptible cartridges on clinical specimens (n=56), and MTBDR*sl* was also done on extract from Xpert rifampicin-resistant cartridges (n=29) and the corresponding culture isolate, when available. Spoligotyping on ~2 µl Xpert extract from clinical specimens and paired culture isolates was done.
MTBDR*plus* on extract from dilution series resulted in high rates of problematic results (TUB-band negative, indeterminate, or false-positive for resistance), whereas almost all MTBDR*sl* on extract from dilutions 10^3-10^6 CFU/ml (C_T≤24.1) were correctly detected as true-resistant or -susceptible. Sequencing resulted in usable data only when Xpert was done at ≥10^5 CFU/ml. MTBDR*sl* on extract from cartridges done on clinical specimens gave low indeterminate rates [n=4/85(5%) and n=6/85(7%)] for the fluoroquinolones and second-line injectable drugs, respectively. Spoligotyping of extract from clinical specimens (n=10) matched their paired culture isolates.
MTBDR*sl* on extract has high diagnostic accuracy and low indeterminate rates. Spoligotyping on extract is feasible. These data have implications for routine DST algorithms and strain typing.

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The Impact of Different Reconstitution Fluids on Mycobacterium tuberculosis (MTB) Susceptibility Testing Results for Linezolid

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Linezolid, classified as a World Health Organization (WHO) group 5 drug, is the first oxazolidinone available for clinical use. With MTB drug resistance on the rise, there is an increasing demand for drug susceptibility (DST) results for the newer drugs. Recent guidelines and recommendations include the critical concentration of 1.0µg/ml for testing linezolid using the Bactec MGIT 960 system, and acceptable minimum inhibitory concentration (MIC) ranges for the quality control (QC) strain, H37Rv. There is however, no commercial drug testing platform, and the lack of standardization and recommendations for in-house drug preparations was the reason for this study.
4 different concentrations of linezolid (0.25, 0.5, 1.0 and 2.0µg/ml) were prepared using methanol as solvent, as recommended by the manufacturer; and sterile deionized water as a diluent (ME-DI method). Two more drug panels were similarly prepared; one using dimethyl sulfoxide (DMSO) and sterile deionized water (DM-DI method); and the other using DMSO as both solvent and diluent (DM-DM method). Two reference MTB strains, H37Rv ATCC 27294 and

H37Ra ATCC 25177, and 4 clinical TB isolates were tested using the MGIT960 and the results compared.
For H37Rv, there was considerable spread in the MICs when tested with linezolid prepared with the ME-DI method (0.5µg/ml, n=2; 1.0µg/ml, n=5 and 2.0µg/ml, n=7). Whereas for the drug sets prepared using the DM-DI and DM-DM methods, the MIC was consistently less than the critical concentration of 1.0µg/ml. Testing of H37Ra was limited in number, but the MIC was 1.0µg/ml by all 3 reconstitution methods. For the clinical isolates, there was no difference in the MICs obtained by both DMSO-based methods.
With the exception of using methanol as a solvent, the MIC results for the QC strains were consistent with previous reports and DMSO would be the preferred solvent for linezolid drug preparation.

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GeneXpert MTB/RIF System in rapid laboratory detection of extrapulmonary tuberculosis – case reports

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Tuberculosis (TB) remains the most serious infectious, and currently even social disease in the world. Over one third of the world population is infected with TB. Due to these grave epidemiologic reasons TB was declared a global threat by the WHO, and its occurrence is subject to obligatory report.
In the Czech Republic (CZ) the number of reported cases of TB is propitious with a low incidence around 5/100,000 inhabitants. In 2002 - 611 new TB cases (552 pulmonary + 59 extrapulmonary), in 2013 - 502 (455 + 47), in 2014 - 512 (461 + 51), in 2015 - 518 (451 + 67), in 2016 - 502 (436 + 66, preliminary results, final results will be available on April 30, 2017).
Geographically, CZ is located in the central Europe - the intersection for migrants from countries with high incidence of TB, including multidrug resistant (MDR) and extensively resistant (XDR) forms. Moreover, the influx of foreigners is on the rise at present. For citizens of CZ they are a hidden threat that may negatively alter the present low incidence of TB in CZ.
Rapid management of TB and mycobacterioses requires early diagnosis, hindrance of their spread, effective treatment, and prevention of the development of drug resistant strains. Early diagnosis is furnished by rapid molecular biologic techniques, of which the quickest in the world is GeneXpert MTB/RIF System, giving results in 2 hours from one specimen, and offering at the same time both the determination of the MTBC together with its sensitivity to Rifampicin (RIF). All biologic specimens, with the exception of blood, can be tested without previous decontamination.
We examined extrapulmonary specimens from patients with nonspecific symptoms: (a) unexplained fatigue and weakness in dialysis patients (urine), (b) nephritis not responding to therapy, (c) unhealing drug-resistant abscesses at different sites: ham (popliteal space), sacral spine, rectum, neck. The GeneXpert MTB/RIF System revealed MTBC in urine (3x), pus with tissues from ham (2x), abscess near sacral vertebra (2x), pus from perirectal abscess (1x), pus from lymph node on neck (1x). The presented case reports show that extrapulmonary occurrence of TB at less usual sites should always be considered.
The GeneXpert MTB/RIF System is irreplaceable in urgent statim examinations, in all cases of suspect TB, and prior to start of biologic therapy.

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Rapid Microarray-based Detection of Rifampicin-, Isoniazid- and Fluoroquinolone Resistance in Mycobacterium tuberculosis using a Single Cartridge

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Introduction: The present test methods for detection of antibiotic resistances in *Mycobacterium tuberculosis* are restricted by their handling requirements and time to result. Thus a simple and suitable test for the point-of-care is needed for the rapid identifications of resistant *M. tuberculosis* strains.
Methods: To be able to discriminate between wild type and important mutations that are associated with rifampicin, isoniazid and fluoroquinolone resistance a test cartridge based on an oligonucleotide array has been designed. The Melt Curve Assay described herein detects 43 different mutations regions in *rpo*B, *kat*G, *gyr*A and the promotor region of *inh*A within 90 minutes. 24 of these mutations are mediating a rifampicin resistance, 8 mutations are associated with an isoniazid resistance and 11 mutations are responsible for a fluoroquinolones resistance. The sensitivity and specificity of the assay were

determined using DNA from *M. tuberculosis* H37Rv in concentration rates of 5 to 60 copies as well as DNA from 5 different nontuberculous Mycobacteria. To characterize the performance of the test a set of 30 sequence-determined *M. tuberculosis* DNA isolates and four crude cell culture extracts in comparison with the genomic DNA of the strains were tested. The clinical value was evaluated with 265 isolates from Swaziland.
Results: The detection limit of the Melt Curve Assay was 24 copies per reaction, no unspecific reaction could be observed. In 97.4 % of cases, results were consistent with the sequencing data. No differences between genomic DNA and crude culture material could be observed.
Conclusion: The Melt Curve Assay is a simple, rapid and accurate and can be applied to detect rifampicin, isoniazid and fluoroquinolones resistant *M. tuberculosis* strains in low-resource settings.

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Evaluation of a Urine-based Rapid Molecular Diagnostic Test with Potential to be used at the Point of Care for Pulmonary Tuberculosis within a Cape Town Cohort

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Background: Current sputum-based diagnostic tests for tuberculosis (TB) do not serve all target populations making optimal diagnosis difficult. TB diagnosis among sputum scarce patients is challenging for timely diagnosis and treatment initiation. Thus, an alternative non sputum based diagnostic test is urgently needed. *Mycobacterium tuberculosis* (*M. tb*)-specific transrenal (Tr) DNA from urine is a potential target for a molecular test to fill the gap in TB diagnosis. The objective of this multi-centre cross-sectional study was to evaluate the value of a novel molecular test using a portable full-automatic analyzer with the potential for usage in resource-limited settings at the point of care.
Material/methods: Spot urine, blood and sputum samples from 428 adults with suspected pulmonary TB (164 HIV positive, 263 HIV negative) were collected at three clinical sites in Cape Town, South Africa. Tr-DNA was isolated from 4 ml of EDTA urine using an in-house method optimized for DNA fragments larger than 38 bp. A rapid double stranded primer-based PCR method targeting a *M. tb*-specific direct repeat region was applied for Tr-DNA identification. The eluate was tested in triplicate using an automated molecular analyser (Alere™ q) with internal controls (positive and negative) included in each test run. The Tr-DNA based test has a short time to result of 45 minutes including DNA isolation.
Results: Of 428 TB suspects, liquid culture was performed on 412, and 175/412 (42.5%) were microbiologically positive. Using liquid culture as gold standard Tr-DNA test showed a sensitivity of 42.86% (75/ 175; 95% CI; 35.42% – 50.54%) and specificity of 88.61 % (210/ 237 ; 95% CI; 83.86% – 92.36%). The Tr-DNA test has a positive predictive value of 73.53%, negative predictive value of 67.74%, positive likelihood ratio of 75.76% and negative likelihood ratio of 67.74%. Among HIV-infected TB patients the sensitivity and specificity were 45.24% and 89.04%, respectively. Combination of smear microscopy and Tr-DNA increased the sensitivity to 83.82% (smear microscopy alone 75.14%) with 96.61% specificity. Reagents required for Tr-DNA isolation are stable at room temperature and no sophisticated laboratory instrumentation is needed.
Conclusions: This multi-center proof of concept study indicates that Tr-DNA has a high specificity and modest sensitivity. Although unsuitable as a stand-alone test, in combination with smear microscopy, it may have the potential to aid TB diagnosis in HIV endemic regions where sputum scarce and extra-pulmonary TB is not uncommon.

P 106

GenoType NTM-DR to identify the subspecies of Mycobacterium abscessus complex and molecular resistance detection from patients with Cystic Fibrosis

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Introduction: The incidence of non-tuberculous mycobacteria (NTM) infection is increasing in patients with Cystic Fibrosis (CF). *Mycobacterium*

avium complex (MAC) and *Mycobacterium abscessus* complex (MABSC) are the NTM species most frequently isolated in these CF patients. The aim of our study is to identify the different subspecies of *Mycobacterium abscessus* complex in patients with cystic fibrosis and to characterize the molecular mechanisms of resistance to macrolides and aminoglycosides by GenoType NTM-DR and their relationship with drug susceptibility testing.

Materials and Methods: We studied 17 *M.abscessus* complex isolated over a period from 2000 to 2016. These MABSC isolates met ATS criteria and were obtained from 17 Cystic Fibrosis patients attended at Hospital Vall d'Hebron.

We used GenoType NTM-DR (Hain Lifescience, Nehren, Germany). Drug susceptibility testing was performed using the broth microdilution method in Sensititre RAPMYCOI plates (Sensititre, Trek Diagnostic Systems, East Grinstead, United Kindom) according to CLSI guidelines. Strains were incubated for 14 days, and clarithromycin MIC was read in 3, 7 and 14 days.

Results: ***Identification of clinical isolates***. Of the total of 17 MABSC studied, we performed GenoType NTM-DR in 13 MABS, 4 of them were unable to be revived after retrieval from storage. There were 10 *M.abscessus* subsp*. abscessus*, 1 *M.abscessus* subsp*. massiliense*; 1 *M.abscessus* subsp *boletii* and 1 was a mixed of subspecies*.* The results of *erm* (41) gene corresponded with clarithromycin (CLM) susceptibility phenotype (100% agreement). The *M.abscessus* subsp*. abscessus* (1) harbouring C28 sequevar and *M.abscessus* subsp*. massiliense* (1) harbouring *erm* (41) deletion were fully susceptible to CLM*.* By contrast, 67% (6/9) isolates with T28 sequevar showed inducible resistance when incubation was extended to 14 days; the remaining 33% (3/9) T28 sequevar and no *rrl* gene mutation showed resistance to CLM (MICs > 8μg/ml) at day 7. The 2 *M.abscessus* subsp*. abscessus* which exhibited phenotypic high level resistance to CLM (MIC>16μg/ml) after 7 days showed mutations in the *rrl* gene.Of the 10 *M.asbscessus* subsp. *abscessus* 2 isolates exhibited phenotypic aminoglycoside resistance with an amikacin MIC of >64 μg/ml, both isolates had mutations in the *rrs* gene. We found that 46%, 61%, 61% of our isolates studied were resistant to tigeciclyne, linezolid and cefoxitin respectively.

Conclusions: The GenoType NTM-DR not only identified the three subspecies of *Mycobacterium abscessus* complex, but also can detect mixed subspecies. The agreement among genotypic (*erm* (41), *rrl* and *rrs*) and phenotypic susceptibility test was 100%.

P 111

Evaluation of an automated method the molecular detection of mycobacterium tuberculosis rRNA in clinical samples

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Introduction-Aim: Tuberculosis is one of the major public health problems, with 1.5 million deaths worldwide in 2015, according to the World Health Organization. The aim of the study was the evaluation of the automated molecular method Real-time Monitor TRCReady-80 (Tosoh Europe N.V., Belgium) for the rapid detection of Mycobacterium tuberculosis complex (MTBC) in clinical samples.

Materials and Methods: As part of the quality management system of the laboratory according to the EN ISO 15189: 2012 standard, each CE/IVD method introduced in the lab is verified using clinical samples. The method was performed according to the manufacturer's instructions and the results were compared to the gold standard method of culture on solid (Löwenstein-Jensen material, LJ) and liquid media (automated system BACTECTM MGIT 960, Becton Dickinson, USA). The accuracy and statistical precision of the method were calculated.

Results: Totally, 109 clinical routine samples were examined between July and September 2016. Ninety samples (83%) were pulmonary and 19 (17%) extrapulmonary (lymph nodes, pleural fluid, pus samples, etc.). The internal quality control of the method was successful. The incidence of tuberculosis in the test population was estimated as 15%. The analytical sensitivity, specificity, positive and negative predictive values of the molecular method compared to culture were 94%, 99%, 94% and 99%, respectively. The verification showed that the method had very high positive and negative predictive values for the detection of MTBC in clinical specimens. Furthermore, and as a proof of principal, the extracted nucleic acid from a number of positive samples was used successfully in the line probe assay MTBDR*plus* (Hain LifeScience, Germany), for the molecular detection of drug resistance.

Conclusions: The process is automated, easy to use and provides faster results in comparison to the conventional nucleic acid hybridization methods.

Additionally the extracted NA can be used for downstream applications, saving time and reducing the handling of contagious clinical samples.

P 119

Prevalence of non-tuberculous mycobacteria in hospital waters of major cities of Khuzestan province, Iran

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Nontuberculous mycobacteria (NTM) are among the emerging pathogens in immunocompromised individuals including hospitalized patients. So,it is important to consider hospitals water supplies as a source for infection. The aim of this study was to determine the prevalence of NTM in the hospital aquatic systems of Khuzestan, Southwest of Iran. Intotal, 258 hospital water samples were collected and examined. After initial sample processing, sediment of each sample were inoculated into two Lowenstein-Jensen medium. The positive cultures were studied with phenotypic tests including growth rate, colony morphology, and pigmentation, with subsequent PCR-restriction enzyme analysis (PRA) and *rpoB* gene sequence analysis. Mycobacterial strains were isolated from 77 samples (29.8%), comprising 52 (70.1%) rapid growing, and 25 (32.4%) slowgrowing mycobacteria. Based on the overall results, *M.fortuitum* (44.1%) was the most common mycobacterial species in hospital water samples, followed by *M.gordonae* (*n*=13,16.8%) and *M.senegalense* (*n*=5,7.7%). Inconclusion, current study demonstrated the NTM strains as one of the major parts of hospital water supplies with probable potential source for nosocomial infections. This finding also help to shedlight on to the dynamics of the distribution and diversity of NTM in the water system of hospitals in the region of study.
Keywords: non-tuberculousmycobacteria, polymerase chain reaction, restriction enzyme analysis, waters amples

P 134

Latent tuberculosis infection (LTBI) in indigenous communities at Puerto Nariño, Amazonas, Colombia

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Introduction: Identification and treatment of individuals with latent tuberculosis (LTBI) is fundamental to achieve World Health Organization Program "End TB Strategy" objectives. 2-3 billion people worldwide are infected with the tuberculosis bacillus of which 5-15% will eventually develop active TB. The main objective of this study was to detect LTBI in the indigenous population of Puerto Nariño, Amazonas, Colombia, using TST (Tuberculin Skin Test). In addition, QuantiFERON®-TB Gold test (QFT®) was applied to individuals diagnosed with active tuberculosis as well as to their contacts.
Methods: 5235 individuals have been screened with TST and tuberculin was repeated 3 months later in 2251 negative patients in order to assess the degree of immunoconversion. TST was applied intradermally and was read between 48 and 72 hours after application. QFT® test was performed in 57 patients with active TB and 294 contacts.
Results: TST was positive at the first screening in 26.0%. Among TST-negative individuals, 876 (38,9%) became TST positive after three months. From of 294 contacts evaluated with QFT®, 15.6% of them were positive and 9.2% had an indeterminate result. From 57 patients with active tuberculosis, 24,6% of them were positive and 8,7 % had an indeterminate result.
Conclusions: PPD test showed that a quarter of the population under study has LTBI. On the other hand, QFT® results showed that 15.6% of contacts were positive for latent tuberculosis as well as 24.6% of active TB patients.
The high proportion of LTBI is probably associated to the high incidence of active TB among inhabitants of the region.

P 144

Provision of systematic testing for active TB in refugees at Centre for Asylum Seekers Accomodation

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Italian data on TB screening among migrants is limited and does not allow for an exhaustive evaluation of different screening approaches. We aim to evaluate a multi-step strategy for screening migrants of the Mediterranean route based on a questionnaire followed, by rapid microbiological diagnosis for active TB.
At the CARA in Mineo (Catania), active TB screening was performed by two clinicians through door-to-door visits. The resident population was screened for symptoms suggestive of TB by means of a standardized questionnaire. Sputum specimens were collected if any of the symptoms was reported. On spot decontamination was performed using OMNIgene-sputum. Sputum samples were tested by smear-microscopy, solid and liquid culture, GeneXpert MTB/RIF (G4) and the new GeneXpertMTB/RIF-ULTRA (under evaluation).
A total of 1613 migrants agreed to participate in the screening so far. Out of 1613 individuals interviewed 485 (30.1%) reported at least one symptom. Among them, 247 underwent microbiological investigations for TB detection. So far eight TB cases detected by either GeneXpert MTB/RIF (G4) or culture were confirmed by further clinical evaluation, leading to an estimate TB incidence rate of 495/100000 person-year (95% CI 210; 890). Results of our microbiological-based screening for active TB are detailed in table 1. Thirteen samples resulted positive to the new version of GeneXpert platform GeneXpert-ULTRA (seven positive at "TRACE" level). Among the ULTRA-positive, four samples were confirmed by culture. Of the five samples tested positive to G4 analysis two of them were also confirmed by culture. All the G4-positive samples were positive at ULTRA analysis too. Only one sample was detected by culture growth while was negative at the rapid molecular diagnosis. Overall ULTRA allowed to detect the 4 culture-positive cases, while G4 missed half of the culture-confirmed samples.
Estimated screening yield for active TB among migrants in CARA centers (434 per 100.000 person) is higher than what reported in literature. Preliminary data showed that ULTRA increases the number of positive compared to G4. More data are needed on how to interpret positive at "TRACE"

P 161

Identification and drug susceptibility testing (DST) of isolates in the M. chelonae / M. abscessus group received at the National Reference Laboratory for Mycobacteria in Norway (NRL) between November 2010 and December 2014

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Rapid growing mycobacteria (RGM) and especially those in the *M. chelonae / M. abscessus* group are becoming more recognized as cause of infection in humans. DST and correct identification of the species and subspecies of *M. abscessus*, are important for choosing the correct treatment. Many laboratories in Norway culture for mycobacteria but send the isolates to the NRL for identification, susceptibility testing and / or retention in biobank. In connection with establishing the recommended method for phenotypic DST of RGM, many of the *M. abscessus* and *M. chelonae* isolates received at the NRL between November 2010 and December 2014 were included in this study. Most of the isolates came from respiratory specimens, from patients of different ages and from different part of the country. In total 51 *M. abscessus* and 10 *M. chelonae* isolates (identified with the LPA from Hain; GenoType Mycobacterium CM) were included and tested with phenotypic and molecular methods. The broth microdilution method was performed in accordance with the CLSI standard from March 2011, and microtiter plates from TREK Diagnostic Systems were used. The isolates were sequenced on the Illumina Miseq platform and Seqman NGen (DNAstar) was used to align fastq reads to a pseudo-genome genbank file consisting of the *M. abscessus* ATCC 19977 genes *rrs*, *rrl*, *rpo*B and *erm* (41). A subgroup of 20 *M. abscessus* isolates were also tested with the new LPA from Hain; GenoType NTM-DR. We compared the phenotypic DST results for amikacin / tobramycin with mutations in the *rrs* gene, and the results for clarithromycin with mutations in the *rrl* gene and the *erm*(41) sequevar. We also compared the identification of

the species and the *M. abscessus* subspecies, by sequencing and the LPAs. The agreement between the phenotypic and molecular DST results, and the identification of species and subspecies with the different molecular methods, was good with a few exceptions.
sence of disputed *rpoB* mutations at positions 526, 527, or 533. Additional discrepancies with resistance phenotypes were only observed with *rpoB* mutations outside the rifampicin resistance determining region. The full data including gene sequencing and the minimal inhibitory concentrations of INH/RIF are currently under final analysis and will be reported in the planned presentation.

P 171

The effectiveness of topical (via nebulizer) use of anti-TB drugs in pulmonary TB patients

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Background: Contomination of trachea and bronchi with MTB and scar stenosis forming is a common complication in sputum-positive pulmonary TB patients. The aim of the work was to determine the effectiveness of anti-TB treatment with the addition of isoniazid sol. and rifampicin lyophilisate sol. inhalations via nebulizer in patients with newly diagnosed pulmonary TB.
Methods: 48 patients with pulmonary TB were divided into two groups - main (MG), n=21 and the control group (CG), n=27. Patients in MG received standard anti-TB chemotherapy + 0.15 g of isoniazid and rifampicin 0.15 g inhaled through a nebulizer daily. Patients in CG received standard anti-TB treatment.
Results: After 1 month sputum conversion was found in 14 (66.7%) patients in MG and 10 (37.0%) - CG, $p< 0.05$. On completion of the intensive phase (IP) sputum conversion was observed in 19 (90.5%) patients of MG and 21 (77.8%) - CG, $p> 0.05$. The average time to sputum conversion was 1,4 ± 0,3 months in MG, and 2,5 ± 0,4 months in CG, $p < 0.05$. Cavities healing occured after completing intensive phase (2 months) in 13 (61.9%) patients in MG and 12 (44.4%) - CG, $p> 0.05$. After completion of treatment course scar stenosis of the bronchi II-III art. diagnosed in 3 (14.3%) patients MG and 17 (63.0%) - CG, $p < 0.05$. The duration of hospital treatment was 2,4±0,4 months in MG and 3,9±0,5 months in CG, $p < 0.05$.
Conclusions: Addition of anti-TB drugs via nebulizer to the standard course of anti-TB chemotherapy in patients with pulmonary TB increases the frequency of sputum conversion on 29.7% for the 1st month of treatment, reduces the incidence of scar stenosis of the bronchi II-III art. on 38.7%.

P 174

Evaluation of colorimetric Microplate Alamar Blue (MABA) and Resazurin Microtiter Assay (REMA) for first line anti-tuberculosis drugs testing of clinical isolates of Mycobacterium tuberculosis

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Tuberculosis (TB) is one of the most important global public health problem.
The increasing incidence of drug resistant TB gives cause for concern around the world. Accurate and rapid drug susceptibility detection is critical for the effective treatment of patients. Conventional drug susceptibility tests are time consuming. Some otomotized systems such as BACTEC 460, MGIT 960 can be determined more rapidly, but the high cost, high volume, lack of high-throughput format and usefulness for mass screening. The colorimetric microdilution assays have been used drug susceptibility of *Mycobacterium tuberculosis*. The advantages of these tests are mainly low cost and the quantitative determination of drug susceptibility against any strain of replicating Mycobacterium tuberculosis to be completed within a week at minimal cost. In this study, using MABA and REMA, a total of 65 clinical isolates were tested against streptomycin, isoniazid, rifampicin, ethambutol and the results compared with those obtained with the gold standard proportion method (PM) on Lowenstein Jensen medium. The sensitivity of INH for MABA and REMA was %95 and %97, % 98 for RIF, 90% and %96 for EMB, and 91% for STR, respectively. In conclusion, MABA and REMA are a simple, rapid, low cost. They can be use an alternative test for drug susceptibility testing and MIC determination.

P 180

Evaluation of the new TBC identification test (TBc ID), a rapid chromatographic immunoassay for the detection of Mycobacterium tuberculosis complex from liquid and solid cultures

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Aim: Tuberculosis (TB) is a disease of major public health concern worldwide, especially in developing countries. Rapid, accurate and simple methods for differentiation of *Mycobacterium tuberculosis* complex (MTBC) isolates from NTM is greatly needed. The BACTEC MGIT 960 system is used to cultivate broth cultures. The Lowenstein Jensen medium is used to cultivate solid cultures. This study was done to evaluate the clinical performance of BD MGIT TBc identification test (TBc ID) and Capilia TB-Neo identification test for rapid identification of MTBC in samples from broth and solid cultures.
Methods and results: A total of 100 Ziehl-Neelsen (ZN) stain-positive MGIT cultures (40) and LJ cultures (60) were tested using the TBc ID tests. The agreement between BD MGIT TBc identification test (TBc ID) and Capilia TB-Neo identification test was 100%.
BD (Becton Dickinson, USA) developed a simple immunochromatographic assay BD MGIT TBc ID test (TBc ID test) using anti-MPT 64 antibodies for rapid differentiation between MTBC and NTM in as little as 15 min. Capilia TB-Neo adopts an immunochromatography method, which can detect the MPB64 antigens specifically produced by the *M. tuberculosis* complex.
Conclusion:TBc ID tests are simple, sensitive, and specific tests for identification of MTBC in liquid and solid medium, do not require a high level of skills, additional specific equipment and gives results in 5-15 min, which provide a good alternative for the rapid identification of *M. tuberculosis* complex in liquid and solid cultures.

P 181

Evaluation of MALDI-TOF MS for Identification of Nontuberculous Mycobacteria Isolated from Clinical Specimens

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Nontuberculous mycobacteria (NTM) are emerging pathogens with increasing prevalence. Especially immunosuppressed patients and people with underlying chronic pulmonary diseases are at risk for NTM infections. Rapid and accurate diagnosis of clinically significant NTM isolates which are identified to the species level is very important as inappropriate treatment may lead to drug resistance or unnecessary exposure to drug toxicity. Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) has been shown as a powerful, inexpensive, and rapid method in the identification of NTM compared to traditional biochemical and molecular techniques. The aim of the present study is to evaluate diagnostic performance of MALDI-TOF MS (Bruker Daltonics, Bremen, Germany) in comparison with PRA-hsp65 method for identification of NTM.
A total of 100 NTM isolates from various clinical specimens between 2004 and 2015 were analyzed in this study. The MALDI-TOF MS analysis was performed with the NTM colonies being inactivated by heating and then zirconia / silica beads were used for mechanical disruption. Formic acid and acetonitrile were used for protein extraction. The PRA method (PCR-RFLP) was based on the amplification of a 441-bp segment of the 65-kDa heat shock protein (*hsp65*) and the restriction enzyme analysis of PCR products using (BstEII and HaeIII).
All of the isolates were identified with PRA-hsp65 method as NTM of which 28 were *M.abscessus* type II, 26 were *M.abscessus* type I, 19 were *M.fortuitum* type I, 6 were *M.lentiflavum* type I, 4 were *M.fortuitum* type II, 3 were *M.peregrinum* type II, 3 were *M.szulgai* type I, 3 were *M.simiae* type I, 2 were *M.gordonae* type III, 1 were *M.gordonae* type IV, 1 were *M.porcinum* type I, 1 were *M.celatum* type I, 1 were *M.chelonae* type I, 1 were *M.kansasii* type I, and 1 were *M.chimaera* type I. Bruker mycobacteria library gave spectral scores higher than 2.0 for 33 (33%), between 1.6 and 2.0 for 61 (61%), and lower than 1.6 for 6 (6%) strains. For 94 strains (94%), the results with MALDI-TOF MS were in good agreement with the results of PRA-hsp65 method. For 6 strains (6%), discordant results were obtained with these two methods. These 6 strains were analyzed with GenoType CM/AS assay (Hain Lifescience GmbH, Nehren, Germany). The results of GenoType CM/AS assay were in concordance with the results of PRA-hsp65 method.
In conclusion, MALDI-TOF MS is a powerful technique capable of performing accurate, rapid, inexpensive, and easy identification of NTM isolates. However sometimes it is inadequate if there is contaminating bacteria or mixed mycobacteria and if some species

are respresented with low numbers of spectra leading to insufficiency of the database. Some further study is required to validate the results in clinical practice.

P 183

Molecular genotyping and drug resistance profiles of Mycobacterium tuberculosis (MTBC) clinical isolates in Adıyaman province of Turkey

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Tuberculosis continues to be a serious problem in the world. Turkey is still among the countries with moderate TB incidence. According to the 2014 World Health Organization (WHO) global TB report, in 2013 the incidence and prevalance rate of TB in Turkey from 20 and 23 cases per 100,000 people. Multi drug resistance rate of new and retreatment cases in 2013 was 0-2,9 % and 12-29,9 % in Turkey. There was no previos data for molecular epidemiology and drug resistance profile in Adıyaman province of Turkey. In the present study, we aimed to determine the molecular epidemiology and drug resistance profile of *Mycobacterium tuberculosis* complex (MTBC) isolates in Adıyaman,Turkey. A total of 46 MTBC strains isolated between Jan 2015-Dec 2016 in Mycobacteriology Laboratory of Adıyaman University, Training and Research Hospital were analyzed by spoligotyping and the 24 mycobacterial interspersed repetitive unit (MIRU)-variable-number tandem-repeat (VNTR) method typing. Susceptibility testing to streptomycin, isoniazid, rifampin and ethambutol was also performed by MGIT 960 system by using SIRE Kit. Within the 46 MTCBC isolates, rifampicin and INH resistance rates were 6.5 % and 8.7% and 4.34 % of multidrug-resistant isolates.Main spoligotype families were identified in 46 isolates. According to MIRU results, 42 patterns (12 orphan and 21 patterns that matched existing MIRU international types in an updated database).

P 184

Characterization of Variants Observed in PncA, RpsA, and PanD and Association to PZA Susceptibility Testing and Pyrazinamidase Activity in MDR/XDR M. tuberculosis Clinical Strains

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Background: PZA is an important first line drug to treat tuberculosis (TB). It is also included in all regimens for the newly developed/repurposed drugs. However, prevalence of PZA resistance continues to rise. Due to issues with growth based PZA tests, molecular diagnostics are being considered as a replacement for these tests. Strong genotypic-phenotypic association is a prerequisite for such a replacement and needs to be carefully evaluated. In the current study, we evaluated this association among 296 mostly XDR-TB clinical isolates from four countries.
Methods: PZA susceptibility was evaluated (Bioproject accession PRJNA353873) through three methods: BACTEC MGIT 960, Wayne's pyrazinamidase (PZase) activity assay, and WGS. Wayne's Assay was used for all isolates that had a discordant result between MGIT and *pncA* genotype. The most noted molecular basis for resistance to PZA has been mutations in three genes *pncA*, *rpsA*, and *panD*. Coding and promoter region mutations in these genes were investigated for association with the results of both phenotypic assays.
Results: We observed high concordance between MGIT and Wayne's assay (95%) in 103 isolates tested. In this study, 202 of 224 (90%) PZA^R (Resistant by MGIT) and 25 of 72 (35%) PZA^S isolates had a mutant PZase.
Four PZA^R isolates with wild type (WT) PZase had a mutation in RpsA or PanD. This only accounts for 18% of PZA^R isolates with WT PZase.
Heterogeneity in *pncA* (but not in *rpsA* or *panD*) had a strong correlation with PZA resistance. 85% of isolates with a heterogeneous PZase were PZA^R.
Lineage analysis revealed that Euro-American PZA^R isolates were more likely (15% of Euro-American as compared to 7% East Asian, 6% Indo-Oceanic, 11% East African) to have a WT PZase and escape molecular detection. We also identified lineage-specific PZase "hot spots". Amino Acids 1-30 and 151-187 in PZase were highly mutated in East-Asian and Euro-American

isolates, respectively.
Conclusions: We observed a relatively high sensitivity in *pncA*. Inclusion of *rpsA* and *panD* did not notably improve sensitivity. Specificity of a *pncA*-based molecular test (65% in our set) is a concern in highly resistant (e.g. XDR) isolates. Our study, like others, assumed that all mutations observed in PZase cause resistance. As such the sensitivity reported in this study is the highest and specificity is the lowest possible. Mutagenesis experiments are needed to confirm the role of all mutations. Exclusion of mutations that do not confer resistance in mutagenesis may improve specificity but also lower sensitivity. The association of heterogeneity in *pncA* with resistance is interesting. Its utility as a diagnostic tool hangs in the balance between the prevalence of this phenomenon and our ability to detect it. Finally, lineage association results indicate potential regional convergent evolution associated with PZA resistance.
Funding: NIAID R01AI105185

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Characterizing Heterogenous DNA Methylation in Mycobacterium tuberculosis Clinical Isolates with Single Molecule Real-Time (SMRT) Sequencing

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Background: Prokaryotic DNA methyltransferases (MTases) methylate specific motifs with high fidelity[1]. DNA methylation serves diverse purposes in prokaryotes. In *Mtb*, it mediates gene expression changes in response to hypoxia[2]. Recent work examining >200 prokaryotic methylomes showed methylation is highly conserved within phyla[2]. Ancestral mycobacteria have all three known MTases, yet clinical isolates exhibit loss of function in each of these MTases[3]. Despite its potential relevance in adaptation to drug and host pressures, DNA methylation has rarely been examined in mycobacteria, and its modulation (heterogeneity) never been studied in *Mtb.* We aim to characterize methylomic heterogeneity in *Mtb* and distinguish between its two forms: 1) genome-wide heterogeneously methylated motif sites (GHMS), suggesting heterogeneous MTase sequence and function; 2) Locus-specific hypomethylation (LSHM), suggesting targeted modulation of gene expression.
Methods: SMRT sequencing, complete *de novo* genome assembly, and determination of methylome of over 100 clinical isolates from high TB-burden countries were done using PacBio's SMRT sequencing and KineticsTools. An in-house pipeline determined heterogeneity patterns from kinetic data. Isolates with GHMS were examined for MTase sequence heterogeneity. Functional consequences of LSHM were determined by transferring H37Rv's annotation onto our genomes using RATT[5].
Results: Heterogeneous methylation appeared in isolates of all four major lineages, but is most varied in EAM isolates. Both forms of heterogeneity were present. LSHM was frequently observed at multiple loci of interest, including *mamA* motifs adjacent to genes implicated in hypoxic response, TAG accumulation, and mce-family proteins, which are associated with virulence. Motifs of the *hsdM/hsdS1* MTase most commonly demonstrated site-specific heterogeneity, whereas genome-wide heterogeneity was most frequent in the *mamB* motif.
Conclusions: Lineage associated methylation heterogeneity is present in two forms, representing two fundamentally different processes. LSHM may reflect inherited methylation patterns, or may result from stochastic epigenetic switches[6]. GHMS may represent an evolutionary point wherein cells lacking a functional MTase are becoming dominant or being phased out. Alternatively, it could indicate programmed phenotypic heterogeneity, a mechanism by which phenotypic heterogeneity manifests from minimal (single-base) indels in an MTase of a subpopulation, radically altering the methylome and creating phenotypically distinct lineages within the colony[6].
Funding: NIAID R01AI105185

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False pyrazinamide resistance in mono-resistant Mycobacterium tuberculosis isolates

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Background: Pyrazinamide (PZA) is a first line key drug in the treatment of tuberculosis (TB). Inside the bacteria, the prodrug PZA is activated by the bacterial enzyme pyrazinamidase (encoded by the *pncA* gene) which converts PZA into its active compound pyrazinoic acid. The

correlation between phenotype and genotype has been shown to be solid, but the phenotypic testing of PZA resistance is associated with some technical difficulties and the challenge of false resistance is well known. Globally, the level of PZA mono-resistance is considered to be low.
Objective: To determine the prevalence of false PZA resistance among *M. tuberculosis* isolates reported as PZA mono-resistant.
Method: 57 clinical *M. tuberculosis* isolates previously classified as mono-resistant to PZA by the clinical TB laboratories were re-tested for phenotypic PZA resistance in the Bactec MGIT 960 system and submitted to *pncA* sequencing. The bacterial samples were isolated in Sweden between 2008 and 2015.
Results: 39 (68%) of the isolates initially reported as PZA resistant turned out as PZA susceptible in the phenotypic re-testing. Sanger sequencing showed that 38 of these samples carried a wild type *pncA* gene while the remaining sample harbored a non-synonymous *pncA* mutation. The results show that the level of false resistance among these isolates is high, but they also indicate a reduction during the last two years of the study period.
Conclusion: Laboratories should always pay extra attention to PZA resistance in otherwise susceptible isolates and sequencing of the *pncA* gene helps to address the issue of false PZA resistance. The improved situation during the last part of the study period coincided with the introduction of PZA in external quality assessments.

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Rapid detection of multidrug-resistant tuberculosis by rolling circle amplification and nanoflares for resource-limited clinics

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Molecular diagnostics targeting nucleic acids is a promising approach for rapid resistance profiling, especially in the case of Tuberculosis, which is a major global health burden. The causative agent, Mycobacterium tuberculosis has infected about 3 billion people, while only 12.2 million people show active infectious symptoms. Failure in accurate detection of correct resistance genotypes in the early-stages leads to the empirical treatment, which contributes to the development of multidrug resistance. Point mutations in the chromosomes of the bacterium commonly recruits drug resistance and remains a challenge for diagnosis and treatment.
Rolling circle amplification (RCA) using padlock probes is one of the most efficient techniques for discrimination of point mutations. For the first time, we have combined RCA with very sensitive nanoflares to produce fluorescence readout signals. Nanoflares are gold nanoparticle-based biosensors which utilize gold mediated quenching for the detection of uniform sized nucleic acid fragments (monomers produced by RCA). Upon hybridization of monomers to the capture oligonucleotides that are conjugated to the surface of nanoflares, the quenched fluorophores (reporters) are released to produce fluorescence. The fluorescent signals developed after reporter displacement is then measured by Relative Fluorescent Units, using a simple fluorimeter. A proof-of-concept assay was established to detect the most clinically significant wildtype codons like rpoB 531 and katG 315 using as low as 20 attomoles from 10 different DNA samples isolated from clinical isolates produces at least 50000 RFUs. By detecting only two codons, we presume to identify about 86% of the drug-susceptibility cases to the major first-line antibiotics like rifampicin and isoniazid, respectively, where the negligible fluorescence signals denote the development of drug resistance. The total turn-around time to obtain sample-to-result is about 70 min, and the test can be easily performed by a semiskilled health care personnel. This viable cost effective and rapid method would assist the clinicians to choose appropriate antibiotics, thereby help to control the spread of drug resistance among the TB infected population.

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