EUROPEAN SOCIETY OF MYCOBACTERIOLOGY

ESM Annual Meeting

25-28 June 2023

Rogner Hotel, Tirana, Albania

Abstracts

www.esmycobacteriology.eu @EsMyco

Invited Speakers

GL03

Target product profiles for tests for tuberculosis treatment monitoring and optimisation.

S Den Boon [1]
1: World Health Organization

Discovery, development and rapid uptake of new tools are critical to substantially reduce TB incidence and reach the global End TB targets. To aid the development of new tests and tools, WHO is developing new target product profiles (TPPs) for tests for TB treatment monitoring and optimisation. These TPPs cover desired tests for three different use case scenarios 1) tests that are done at treatment initiation to identify patients who should be treated with a different or shorter TB regimen; 2) tests that are done during treatment to identify patients with a poor response to treatment who might benefit from a different or longer (optimised) TB treatment regimen, adherence support interventions or adjunctive therapies, or undergo further testing (e.g. for drug resistance); and 3) tests done at the presumed end of treatment to identify patients who should stop TB treatment ("test of cure"). The TPPs identify minimum and optimal targets for the following characteristics (some of which are common to the three use cases and some of which are unique): assay/instrument design, sample throughput (for instrument-based assays), operational environment, environmental impact, maintenance (for instrument-based assays) and quality control, target setting, target user, training and education, turnaround time, results, cost of test (including cost of reagents and consumables), cost of instrument, target population, sample type (if a clinical sample is required), sensitivity, specificity, timing, and frequency. These TPPs are expected to serve as an important tool to set research and development targets for funders and developers.

GL10

The X(DR)-Files – I want to believe

C U Köser [1]
1: University of Cambridge

Following the endorsement of BPaL(M) by WHO, the need to scale up the capacity for antimicrobial susceptibility testing (AST) for bedaquiline, delamanid and linezolid has become pressing. However, AST for bedaquiline and delamanid, as well as the related compounds clofazimine and delamanid, are challenging due one or more of the following factors: a large spectrum of resistance mutations; a low prevalence of resistance; differences in the intrinsic susceptibility; disagreements about the meaning of 'resistance'; epistasis; heteroresistance; insufficiently validated phenotypic AST methods; and modest increases in the minimum inhibitory concentrations conferred by some resistance mechanisms. These issues can result in systematic, random or cut-off errors that are exacerbated by the lack of guidance for mitigating them, which risks undermining the trust of clinicians in AST and increases the likelihood of baseline and acquired resistance going undetected.

GL11

Whole genome sequencing for tuberculosis: it works, how do we get it used more widely?

A E Brankin [1] D Adlard [1] J Westhead [1] S Valley Omar [2] P W Fowler [1]
1: University of Oxford 2: National Institute for Communicable Diseases

Whole genome sequencing is becoming a key method for inferring which drugs can be used to treat tuberculosis infections. To reduce sequencing errors, bioinformatic tools are usually setup so majority of reads are required to call a genetic variant; one unfortunate consequence of this approach is that minor populations are not usually detected.

By analysing 8,138 samples from the CRyPTIC project we show how allowing just two reads supporting A90V or D94G in *gyrA* increases the sensitivity of moxifloxacin resistance prediction from 85.4% to 94.0% with no significant change in specificity (similar result for levofloxacin). Interestingly the proportion of the reads containing the resistant variant does not correlate with the MIC [1]. These results are important since the fluoroquinolones are increasingly being included in treatment regimes yet, despite it being generally accepted that we understand their resistance mechanism, their sensitivities have remained lower than those of first-line drugs like rifampicin.

Such observations are, however, useless unless they can be put into practice: we will therefore describe how such rules can be incorporated into resistance catalogues parseable by piezo. This software can then included in a larger bioinformatic workflow, gnomonicus, which consumes a VCF file and a catalogue and returns the antibiogram to the user.

Finally, we will examine evidence from the CRyPTIC dataset that minor populations play a role in conferring resistance to other drugs and share some preliminary data examining the impact of minor populations on bedaquline resistance.

1. Brankin AE, Fowler PW (2023). *JAC-Antimicrobial Resistance* 5:dlad039
2. https://github.com/oxfordmmm/piezo

GL12

Animal models to study tuberculosis

P J Cardona [1 2 3 4]
1: Hospital Universitari "Germans Trias i Pujol" 2: Unitat de Tuberculosi Experimental. IGTP
3: Universidat Autonoma de Barcelona 4: CIBERES

The use of laboratory animals for experimental modelling in tuberculosis (TB) has been instrumental in understand its natural history. One essential question has been the understanding of the mechanisms of latent tuberculosis infection (LTBI). Murine strains (C57BL/6 and DBA/2) were instrumental in find a key cell, the foamy macrophage, to demonstrate the constant drainage of dormant bacilli towards the bronchial tree, and the possibility of endogenous reinfection of the parenchyma. This explains the usefulness of isoniazid in the chemoprophylaxis. On the other hand, large mammals like minipigs, provided evidence on the role of interlobular septa, crucial for

respiration, and their capacity to rapidly encapsulate minimal granulomatous lesions. This process made it impossible to understand how the progression from LTBI to active TB could occur, as it requires a significant enlargement of the lesions. It was another murine strain (C3HeB/FeJ) which gave a clue on this, showing that a strong neutrophilic infiltration, extracellular bacillary growth, induction of daughter lesions and finally the coalescence of all of them were required for the progression towards active TB. This model has been recently validated using macaques as laboratory animals. All these mechanisms help to understand the difficulty in finding better diagnostic approaches, because a part from radiographic methods, it is difficult to find a biomarker that can indicate *Mycobacterium tuberculosis* infection in upper lobes, where the majority of active TB lesions take place.

GL14

Phenogenomic analyses: linking mycobacterial behaviours to molecular mechanisms

L Boeck [1 2]
1: Department Biomedicine, University of Basel 2: Pulmonary Medicine, University Hospital Basel

Mycobacterium abscessus is a newly emergent multidrug-resistant pathogen causing increasing numbers of often incurable pulmonary infections, particularly in patients with Cystic Fibrosis. However, the genetic determinants underlying *M. abscessus* infection, virulence and how *M. abscessus* survives long periods of multidrug treatment are poorly understood.

To address this gap, we developed phenogenomic analyses, a multi-modular strategy aimed to capture the complexity of genome-wide associations. We phenotyped 331 isolates across 58 phenotypic dimensions including planktonic growth, antibiotic resistance, macrophage infection, *in vivo* infection of *Drosophila melanogaster* and clinical outcomes. Our analysis revealed isolate clusters with distinct phenotypic traits which are related to clinical outcomes. We combined conventional genome-wide association studies with computational modelling to define causal genetic variants and the study of variant coevolution to identify epistatic gene networks. Several revealed mechanisms and networks, including previously unknown virulence factors, were validated by CRISPR-based silencing.

Additionally, we explored antibiotic tolerance, which allows increased bacterial survival during normally lethal conditions. To assess antibiotic killing at large scale, we established Antimicrobial Single-Cell Testing (ASCT), a single-cell imaging-based technology. We found that drug tolerance is defined by the genetic background but different from drug resistance. Moreover, drug tolerance was an independent predictor of clinical outcomes, highlighting its potential use as a novel marker of antibiotic activity. Finally, by applying phenogenomic analyses to drug tolerance phenotypes in *M. abscessus*, we identified several molecular mechanisms that likely confer multidrug tolerance.

GL15

The organoid revolution to assess mycobacterial pulmonary infections

S A Leon-Icaza [1] S Bagayoko [1] R Vergé [1] N Iakobachvili [2] C Ferrand [1] T Aydogan [3] C Bernard [1] A Sanchez Dafun [7] M Murris-Espin [4] J Mazières [4] P J Bordignon [1] S Mazères [7] P Bernes-Lasserre [5] V Ramé [5] J M Lagarde [5] J Marcoux [7] M P Bousquet [7] C Chalut [7] C Guilhot [7] H Clevers [6] P J Peters [2] V Molle [3] G Lugo-Villarino [7] K Cam [7] L Berry [3] E Meunier [1] C Cougoule [1]

1: Immune Detection and Elimination of Pathogens, Institut de Pharmacologie et de Biologie Structurale (IPBS), Université de Toulouse, CNRS, UPS, Toulouse, France 2: M4i Nanoscopy Division, Maastricht University, Maastricht, Netherlands 3: Laboratory of Pathogen Host Interactions (LPHI), Université Montpellier, CNRS, Montpellier, France 4: Service de Pneumologie, Hôpital Larrey, CHU de Toulouse, Toulouse, France 5: Imactiv-3D SAS, 1 Place Pierre POTIER, 31100 Toulouse, France 6: Oncode Institute, Hubrecht Institute, Royal Netherlands Academy of Arts and Sciences and University Medical Center, Utrecht, Netherlands 7: Institut de Pharmacologie et de Biologie Structurale (IPBS), Université de Toulouse, CNRS, UPS, Toulouse, France

Mycobacterium abscessus (Mabs) is an opportunistic pathogen whose incidence and prevalence have risen in recent years owing to the increasing of immunocompromised and vulnerable individuals, such as cystic fibrosis (CF) patients. Nevertheless, the models for determining the host and bacterial factors that lead to Mabs pathology in CF patients are still rudimentary. In our project, we applied the human airway organoid (AO) technology to decipher early events during colonization and infection of the lung epithelium by Mabs, as well as the efficacy of potential new treatments.

First, we show that airway organoids derived from CF patients (CF-AO) present a thicker epithelium, accumulate mucus, and undergo increased oxidative stress, lipid peroxidation, and cell death, key features of CF disease. Next, we reveal that in Mabs infected-AOs the smooth morphotype (S) forms aggregates, while the rough (R) form cord serpentines. Along Mabs infection, the redox pathways were the most dysregulated in the organoids.

Finally, we demonstrate that Mabs take advantage of the exacerbated oxidative environment in the CF-AOs to thrive. By boosting detoxification pathways with NRF2 agonists (master regulator of the antioxidant pathways), such as sulforaphane, the basal levels of reactive oxygen species (ROS) and cell death in CF-AOs decreased via the enrichment of the NQO1 mRNA pool, those contributing to better control of Mabs growth. This opens new possible druggable pathways to better control Mabs infection.

GL17

Global trends of pulmonary infections with nontuberculous mycobacteria

V N Dahl [1 2 3]
1: Department of Infectious Diseases, Aarhus University Hospital, Aarhus, Denmark 2: Center for Global Health, Aarhus University (GloHAU), Aarhus, Denmark 3: International Reference Laboratory of Mycobacteriology, Statens Serum Institut, Copenhagen, Denmark

Non-tuberculous mycobacteria (NTM) may cause progressive pulmonary disease (NTM-PD), especially in patients with underlying structural lung damage. NTM have been reported increasingly worldwide, such as in population-based data from North America, Europe and East Asian. Smaller single-center studies have also supported increasing trends of NTM pulmonary infection among patients considered of tuberculosis (TB) in TB high endemic countries.
In this presentation, a recent systematic review on the global trends of NTM pulmonary infection will be presented and global trends of NTM will be discussed. Finally, population-based data from Denmark through 31 years will be presented.
Current NTM data seem to indicate clear increasing trends in most countries and regions, at least for absolute numbers of infection, but also for relative numbers (i.e., when taking population sizes into account). When evaluating trends, access to and quality of diagnostics, differences among age groups, species, incidence vs. prevalence, disease manifestation and comorbidities, among others, should be taken into account, and may explain some of the observed geographical differences.

Oral Presenters

OR01

Understudied and overlooked: Characterizing *Mycobacterium orygis*

S N Danchuk [1 2 3] S C Duffy [1 2 3] F A McIntosh [2 3] J Y Dubé [2 3] A Lupien [2 3] P Joubert [4] M A Behr [1 2 3 5]
1: Department of Microbiology and Immunology, McGill University 2: Research Institute of the McGill University Health Centre 3: McGill International TB Centre 4: Institut Universitaire de Cardiologie et de Pneumologie de Quebec 5: Department of Medicine, McGill University Health Centre

Mycobacterium tuberculosis (*M. tb)* is the causative agent of human tuberculosis (TB) whereas *Mycobacterium bovis* and *Mycobacterium orygis* are the cattle-associated lineages. Unlike *M. bovis*, there is limited knowledge of *M. orygis* and its capacity to infect and cause disease. To address this gap, we developed an experimental *M. orygis* infection model. We compared the infection outcome in C57BL/6 mice after aerosol exposure to *M. tb* H37Rv and *M. orygis* 51145. Unlike *M. tb*, where experimental infection is monitored at days 21, 42 and 84, the *M. orygis* group experienced mortality as early as 21 days. Macroscopically lungs showed significant inflammation and granuloma-like structures; histopathology showed extensive neutrophilic consolidation and destruction of airways. *M. tb* and *M. orygis* bacterial burden at this time point were comparable. We determined that at ~150 bacteria, the *M. orygis* survival has a bimodal distribution, with early (~4 weeks) and late (~4 months) waves of mortality. While *M. orygis* had a similar infection time-course as *M. bovis* Ravanel, to achieve this same distribution using *M. tb* we required > 1200 CFUs. Lastly, we were also able to delay mortality following subcutaneous vaccination with *M. bovis* BCG from ~4 weeks to ~4 months. In synopsis, *M. orygis* is a unique lineage of the MTBC. Despite a distinct genomic identity from *M. bovis*, *M. orygis* has similar hyper-virulence following experimental murine infection. Further characterization of animal-associated and human-associated disease is required to better understand this overlooked pathogen.

OR02

Mycobacterial extracellular vesicles (MEVs) as a novel option in bladder cancer therapy

R Sorrentino [1] P Miotto [1] C Venegoni [2] F Pederzoli [2] V Murdica [2] A Salonia [2] D M Cirillo [1] M Alfano [2]
1: San Raffael Scientific Institute 2: Urological Research Institute (URI-HSR)

The intravesical instillation of the BCG vaccine (attenuated *Mycobacterium bovis*) after the transurethral resection of bladder tumour is the gold-standard treatment for patients with high-risk non-muscle invasive bladder cancer (NMIBC). Nevertheless, only about 50% of patients show a complete response to BCG treatment, and mild to severe side effects are very common.

Extracellular vesicles (EVs) from mycobacteria (MEVs) have recently been shown to play a role during mycobacteria infection.

In this study, we characterized MEVs and their effect on bladder cancer cell lines (BCaCL).

MEVs were isolated from BCG and *Mycobacterium tuberculosis* H37Rv stationary-phase cultures and purified by size exclusion chromatography, next characterized physiochemically and expression of mycobacterial markers. Subsequently, different grade bladder cancer cell lines (BCaCL) were treated with 5 and 10 µg of MEVs or infected with viable BCG for 72 hr. BCaCL response was assessed by calcein assay and ELISA cytokines quantification.

Western Blot confirmed the purity of MEVs (LAM and LpqH positive; eukaryotic markers negative). Live BCG and BCG-MEVs displayed a degree of cytotoxic effect, but only H37Rv-MEVs showed a statistically significant cytotoxic against different BCaCL ($p<0,05$). Cytokine profiling highlighted a higher immunomodulatory capability of BCG-MEVs and H37Rv-MEVs compared to BCG infection (higher modulation of TNFα, IL-6, and IL-12).

Our results support the hypothesis that MEVs can mimic the effect of BCG while overcoming the side effects related to the use of viable bacteria for the treatment of NMIBC.

OR03

Mycobacterial interactions promote Vγ9Vδ2 T cells to target and kill cancer cells

I C Schiavi [1] M Bodman-Smith [1] L Ridgley [2] T Bull [1]
1: St. George's University of London 2: AstraZeneca

Inoculation of live BCG into melanoma or bladder cancer sites promotes host Vγ9Vδ2 (Vδ2) T cell infiltration and disease regression. Efficacy can be enhanced by priming with bisphosphonates, such as zoledronic acid (ZA), suggesting engagement of phosphoantigens and BTN3A1-Vδ2TCR receptors. Unfortunately, repeated dosing with BCG can be detrimental, resulting in granulomas and is contraindicated in immunosuppressed individuals. Additionally, ZA is rapidly sequestered in the bone. Defining components driving these phenomena could offer focussed, practical and safer application of this form of cancer immunotherapy. We aim to derive capacity of mycobacterial lipid fractions to activate human Vδ2 T cell populations driving cancer cell killing.

Lipids from diverging morphological phenotypic cultures of *Mycobacterium vaccae* and BCG were fractionated using chloroform, methanol, and water. Vδ2 T cells were exposed to live mycobacterial cultures or corresponding extracted lipids, expanded for 13 days then co-cultured with tumour cell lines or ZA-primed tumour cell lines. The degree of Vδ2 T cell directed cytotoxicity was determined using live/dead staining and FACS.

Live mycobacteria exhibited a diverse interactivity with Vδ2 T cells related to morphotype, allowing them to expand, target and kill cancer cells. Killing was enhanced by ZA-priming of target cell populations. Lipids extracted from the same morphotypes similarly activated and expanded Vδ2 T cell populations but generated a significantly greater cancer cell killing efficiency than stimulation by live bacteria alone. Additionally, this effect was independent of ZA-priming of target cells. Interaction of mycobacterial lipid fractions with Vδ2 T cells promotes their ability to kill cancer cells.

OR04

Uncovering epigenetic changes in early-stage MTBC-infected macrophages

J Schoenfeld [1] T Dallenga [1] S Homolka [1 2] S Niemann [1 2]
1: Research Center Borstel, Germany 2: National Reference Center for Mycobacteria, Borstel, Germany

Despite immense efforts to end tuberculosis, infections with strains of the *Mycobacterium tuberculosis* complex (MTBC) still cause extensive global mortality and morbidity. Previous studies showed that MTBC infection can lead to detrimental epigenetic modifications of host cells, resulting in immune exhaustion. Macrophages play a critical role in host defense against MTBC and understanding epigenetic alterations in response to infection can provide valuable insights into host-pathogen interactions and immune response.

In our study, we aim to investigate changes in the epigenetic makeup of human macrophages, such as DNA methylation, gene expression, and histone modifications, during early-stage infection with different MTBC strains. Studying epigenetic changes in infected macrophages requires a robust protocol allowing for high quality DNA and RNA extraction while sterilizing infectious samples.

We developed a protocol that ensures biosafety while maintaining the integrity of nucleic acids. To achieve this, we tested a variety of DNA/RNA extraction protocols for their capacity to efficiently inactivate *Mycobacterium tuberculosis* and analyzed the resulting nucleic acid quality. To our surprise, even commercially available reagents did not lead to a complete inactivation of the samples. The final protocol developed, allows for high quality DNA/RNA extraction, while all sterility controls remained negative.

In conclusion, our study provides a robust protocol for studying the epigenetic alterations that occur in infected macrophages while ensuring sterility and preserving DNA and RNA integrity. This protocol can be valuable for future studies aimed at understanding the complex interplay between MTBC and host cells.

OR05

Molecular evolutionary analysis of a clade of closely-related Delamanid-resistant *Mycobacterium tuberculosis* (*M.tb*) strains from Eastern Europe and Central Asia.

K R Wollenberg [1] M Harris [1] A Gabrielian [1] N Ciobanu [2] V Crudu [2] N Shubladze [3] A Rosenthal [1]
1: National Institute of Allergy and Infectious Diseases 2: Institute of Phthisiopneumology 3: National Center for Tuberculosis and Lung Diseases of Georgia

Drug-resistant (DR) tuberculosis is a global health issue. Highly resistant cases require the development of novel antibiotics, such as Delamanid. To study molecular mechanisms of developing drug resistance, NIAID TB Portals Program had sequenced and analyzed more than 3300 genomes of *M. tuberculosis*. Among these, we identified a phylogenetically robust clade of Lineage 2 samples with the Delamanid resistance variant *ddn Trp88Stop*. Twelve of these samples were from Georgia, five were from Moldova, and one each were from Ukraine and Azerbaijan. The literature provided six additional samples from Tajikistan and eight from Moldova with this DR variant. These samples clustered within the previously-identified clade of TB Portals database Lineage 2 samples. Additional analysis of sample dates and sequence diversity indicated potential transmission clusters within this clade. Two additional samples from Georgia and three from Moldova were from lineage 4 and had this Delamanid resistance mutation. Among all samples some relationships were consistent with the spreading of a specific Delamanid-resistant strain of *M.tb* across international borders in this region. Other samples in this analysis could be the result of parallel acquisition of delamanid resistance in co-circulating, closely related clones. The distribution of the *ddn Trp88Stop* mutation across taxonomic lineages demonstrates independent acquisition of novel drug resistance. We demonstrated that Delamanid resistance is spreading through central Asia and eastern Europe by a combination of the transmission of resistant clones and the rapid acquisition of a specific drug-resistance mutation, further supporting the need for adherence to WHO recommendations for DR-TB treatment.

OR06

Bayesian probability of bedaquiline resistance to guide rifampicin-resistant tuberculosis treatment

P HT Tu [1] D Z Anlay [1 2] R Kessels [1 3] E Riviere [1] S Abrams [1 4] T Decroo [5] A Van Rie [1]
1: University of Antwerp 2: University of Gondar 3: Maastricht university 4: University of Hasselt 5: Institute of Tropical Medicine Antwerp

Bedaquiline is a core drug for rifampicin-resistant-tuberculosis (RR-TB) treatment. Accurate prediction of bedaquiline-resistant phenotype from genomic data remains challenging. We used a Bayesian approach to combine expert opinion and phenotype-genotype data on 756 isolates and estimate the posterior median probability of bedaquiline resistance (PBR) and 95% credible intervals (CrIs) for variants in bedaquiline candidate resistance genes. To assess how the novel PBR concept may be used in clinical care, we performed a discrete choice experiment with 45

experienced physicians. The median PBR was highest for missense mutations in *atpE* (60.8%, 95% CrI 44.0-76.1) and nonsense mutations in *Rv0678* gene (55.1%, 95% CrI 27.3-80.7) and lowest (≤3%) for synonymous mutations in *atpE* and *Rv0678* and missense mutations in *Rv0679*and *pepQ*. PBR played the most important role in determining the physician's treatment decision, followed by patient's response to treatment at 1 month and the strain's resistance profile. The influence of the PBR on physicians' decisions depended on the 95% CrI width and history of exposure to bedaquiline. Physicians were most likely to stop bedaquiline when the PBR was >75%, continue bedaquiline when the probability was <50%, and strengthen the bedaquiline-containing regimen when PBR was between 50% and 75%. Probability of resistance (and 95% CrIs) is a novel promising approach to communicate genomic information when accurate binary classification is not yet possible. Developing a clinical decision support tool for PBR, together with ensuring access to next-generation sequencing has the potential to improve the treatment outcomes of RR-TB.

OR07

Evolution of resistance to new drugs in high tuberculosis burden country.

N Ciobanu [1 2] A Codreanu [1] N Turcan [1] E Noroc [1 2] D Chesov [1 2] D Rusu [1 2] A Donica [1] V Crudu [1 2]
1: Institute of Phthisiopneumology, Chisinau, Moldova 2: State University of Medicine and Pharmacy, Chisinau, Moldova

Background: Drug resistance remains a significant challenge in TB treatment and control programs. New agents have shown promising results for MDR/XDR-TB patients, improving the culture conversion rate, reducing mortality. However, in recent years, an increasing number of cases with acquired resistance to new anti-tb drugs have been reported.

Aim: to assess the emergence of resistance to the new antituberculosis drugs: bedaquiline, clofazimine, delamanid and linezolid.

Method: A total of 1037 MDR-TB patients who underwent treatment between January - December 2020 in 3 TB hospitals from Moldova were examined. M.tuberculosis isolates were tested for resistance to fluoroquinolone, bedaquiline, clofazimine, delamanid and linezolid by phenotypic (BACTEC MGIT 960) and molecular (Hain) methods.

Results: Among the 534 new TB cases and 503 previously treated cases, correspondingly 79.1% (n=422) and 60.4 (n=304), were susceptible. Pre-XDR TB was detected in 11.6% (n=62) new cases and 24.5%% (n=123) previously treated cases. XDR TB was present in only 2 patients among new cases and 7.5% % (n=38) previous cases. Any BDQ resistance – 0.4% new cases and 5.7% previously treated cases, any LZ resistance – 4.2% previously treated cases, any CLF resistance – 1.9% previously treated cases, any DLM resistance – 0.9% new cases and 3.8% previously treated cases.

Conclusions: The emergence of BQ&DLM resistance is alarming as it may lead to the rapid loss of these new drugs. The development of molecular assays for systematic surveillance of new anti-TB compounds is urgently needed to monitor the emergence of resistance and should be a higher priority when new drugs are introduced.

OR08

Drug-specific differential culturability in diverse strains of *Mycobacterium tuberculosis*

S Borrell [1 2] V F.A March [1 2] S Gagneux [1 2]
1: Swiss Tropical and Public Health Institute-University of Basel 2: University of Basel

Recent years have highlighted the importance of drug-pathogen interactions on clinical outcomes in microbial infections. This includes assessing phenomena such as tolerance, persistence and more recently, resilience on the clearance of bacteria by antibiotic chemotherapy.
In *Mycobacterium tuberculosis*, it has previously been shown that differentially culturable bacteria often grow poorly on nutrient rich solid media but are detectable using other culture methods due to metabolic changes in oxidative stress. We hypothesized that antibiotic treatment itself can induce oxidative stress in Mtb, resulting in differential culturability, which has implications for assay design when investigating drug-pathogen interactions.

To investigate the effect of antibiotic treatment on *Mtb* culturability, we subjected a set of clinical strains from different phylogenetic lineages to high-dose short-interval exposure to Rifampicin (RIF), Isoniazid (INH) or RIF and INH in combination at clinically relevant doses for 72 hours. After treatment, drugs were washed out by two cycles of centrifugation and resuspension in 7H9. Survival was assessed by plating on 7H11 and counting colony-forming units (CFUs), and recovery was assessed by allowing recovered bacteria to regrow in falcons tubes shaking at 37°C shaking at 140rpm.

Interestingly, our investigation revealed a disparity between INH-treated cultures and RIF-treated cultures in terms of recovery in broth compared to solid media. RIF-treated cultures indicated higher survival compared to INH when considering CFUs, however, INH-treated cultures recovered faster in broth.

Our initial investigation has indicated that drug-specific action on Mtb can affect subsequent culturability. This requires further evaluation to inform relevant and reliable experimental design when investigating drug-pathogen interactions.

OR09

Prognosis and Prevention of Antibiotic Resistance in *Mycobacterium tuberculosis*: the Isoniazid (INH) Case Study

S J Valafar [1]
1: Chicago Medical School

Drug resistant Tuberculosis (TB) continues to be a major global concern. The ability to predict emergence of antibiotic resistance is clinically important, yet still illudes us after years of molecular and evolutionary assessment of *M. tuberculosis*. INH resistance (INH^R) is commonly first to emerge among anti-TB drugs. As such, the hope is that prognosis of imminent INH^R and changing treatment course, would prevent emergence of resistance to INH but also to other subsequent drugs.

To assess the feasibility of INH^{R} prognostics, I considered 21,595 clinical isolates in two projects. The first set was curated by my systematic review in 2021 (PMCID: PMC8092511). It contained 9,306 clinical isolates (5,804 INH^{R}, 3,502 INH^{S}) from 31 countries. The second set was collected globally by the CRyPTIC consortium (PMCID: PMC9363010) and included 12,289 clinical isolates from 27 countries on five continents. In both data sets, the three most frequently mutated loci continue to be *katG*315, *inhA*-15, and *inhA*-8. Time-course samples from the CRyPTIC dataset was used to develop prognosis hypotheses. The combination of the two sets were used to estimate the prognostic power of each variant. While the prognostic power of most mutations are low, I report two mutations that combined provide acceptable prognostic value. When observed in INH-susceptible isolates, they provide a 71% probability of imminent emergence of *katG*315, and 40% for emergence of *inhA*-15. This information can be used to predict emergence and change treatment regimens in time to avoid INH^{R} emergence.

OR10

The MAGMA platform for global and equitable WGS-guided management of drug resistant TB and TB control

V Rennie [2] T H Heupink [2] L Verboven [2] J Membrebe [3] A G Paytuví [1] R Malinverni [1] É M Martínez [1] W Sanseverino [1] A Van Rie [2]
1: Sequentia Biotech SL, Calle Comte d'Urgell 240, 08036 Barcelona, Spain 2: Family Medicine and Population Health (FAMPOP), Faculty of Medicine and Health Sciences, University of Antwerp, Wilrijk, Belgium 3: Interuniversity Institute for Biostatistics and statistical Bioinformatics (I-BIOSTAT), Data Science Institute, Hasselt University, Hasselt, Belgium

Whole genome sequencing (WGS) holds great potential for the management and control of tuberculosis and is increasingly implemented in high income countries. In these settings, laboratories mostly use non-standardized in-house bioinformatics pipelines. Commonly used freely available bioinformatics pipelines (such as MTBSeq and UVP) were developed for retrospective rather than real-time analyses and struggle to accurately analyze clinical samples characterized by high contamination and low *Mtb* burden. The MAGMA (Maximum Accessible Genome for Mtb Analysis) pipeline was specifically developed for analysis of WGS data obtained from DNA extracted directly from sputum samples or from early positive primary liquid *Mtb* cultures. However, the interpretation of MAGMA pipeline outputs, such as drug resistance profile and phylogenetic information on clusters and recent transmission, requires a level of expertise that is often lacking in settings with high TB burden. To make WGS-guided care globally implementable, bioinformatics pipelines must produce outputs that are trustworthy, easily interpretable, and actionable by clinicians caring for TB patients, reference laboratories, and public health professionals working in TB control programs. The MAGMA platform aims to create standardized, user-friendly, actionable outputs. These outputs can be used for precision public health (targeted contact and source investigation), resistance surveillance (prevalence of resistance and emergence of new resistance conferring variants), and precision medicine (personalized treatment for drug resistant TB). Once developed and field-tested, the MAGMA platform could remove the current expertise and infrastructure bottlenecks and enable equitable global implementation of WGS-guided TB control and drug resistant TB management.

OR11

Analysis of the limited Mtb pan-genome reveals potential pitfalls of pan-genome analysis approaches

M G Marin [1] M Behruznia [2] C Meehan [2 3] M R Farhat [1 4]
1: Department of Biomedical Informatics, Harvard Medical School 2: Department of Biosciences, Nottingham Trent University 3: Unit of Mycobacteriology, Institute of Tropical Medicine 4: Pulmonary and Critical Care Medicine, Massachusetts General Hospital

It can be difficult to benchmark the accuracy of pan-genome analysis methods given the high diversity of many bacterial species and the common lack of ground truth datasets with complete assemblies. *Mycobacterium tuberculosis* (*Mtb*) is a highly clonal bacterium with no horizontal gene transfer, enabling evaluation of the performance and tradeoffs of existing pan-genome analysis approaches. In this work we used a diverse set of 158 *Mtb* isolates with complete genome assemblies generated using a hybrid long- and short-read sequencing. Across the 22 total different parameters of pan-genome analysis approaches we observed a surprisingly large range of accessory genome size predictions, with predictions ranging from 314 to 2951 genes. To complement this analysis, we built a *Mtb* pan-genome graph to detect accessory regions at the nucleotide level. From our pan-genome graph approach we find that only 5.5% (70 kb) of the variation represented novel sequence content relative to the H37Rv reference genome. We demonstrate that pan-genome analysis predictions depend heavily on the choice of software, and the quality of the dataset used. If pan-genome analysis methods are not used properly they can greatly overinflate the predicted accessory genome. We find pan-genome graphs are better for identifying loss or gain of new sequences, in comparison to common coding sequence centric approaches. Our analysis of a *Mtb* pan-genome graph analysis at the nucleotide level further supports that the *Mtb* genome is evolving in a clonal manner and has a limited accessory genome.

OR12

TB-ANNOTATOR: A scalable web application that allows in-depth analysis of very large sets of publicly available Mycobacterium tuberculosis complex genomes.

G Senelle [1 2] C Guyeux [1 2] E Cambau [3 6] G Refrégier [4 5] C Sola [3 4]
1: FEMTO-ST Institute, UMR 6174 CNRS, DISC Computer Science Department, Univ. Franche-Comte (UFC) 2: Université de Bourgogne Franche-Comté 3: INSERM UMR1137 4: Université Paris-Saclay 5: UMR 8079 CNRS-UPsay-AgroParisTech 6: APHP, GHU Nord site Bichat, Service de mycobacteriologie specialisee et de reference

Tuberculosis continues to be one of the most threatening bacterial diseases in the world. Since the beginning of the NGS era, there are more than 160,000 Short Read Archives (SRAs) of *Mycobacterium tuberculosis* complex in the databases. Gathering this high amount of data could help better understanding this bacterium and fighting against tuberculosis. In addition, after gathering, it is important to be able to study, in its entirety and in-depth, this important mass of data. We developed the “TB-Annotator” web application that combines a database containing at the time of writing 102,000 SRAs (after checking their quality). We present a fully featured

analysis platform to explore and query such a large amount of data. The objective is to present this platform tool centered on the key notion of exclusivity, to show its numerous capacities (detection of single nucleotide variants, insertion sequences, deletion regions, spoligotyping, etc.) and its general functioning. We compared TB-Annotator to existing platform tools for the study of tuberculosis, and showed that its objectives are original and have no equivalent at present. The database on which it is based will be presented, with the numerous advanced search queries and screening capacities it offers, and the interest and originality of its phylogenetic tree navigation interface will be detailed. We will end this presentation with examples of the results made possible by TB-Annotator, followed by avenues for future improvement.

OR13

Mycobacterium tuberculosis infecting *Drosophila melanogaster*: first insights of the new latent tuberculosis infection model.

M Vidal [1 2 3] P Soldevilla [1 2 3 4] M Arch [1 3] E Fuentes [1 3] J Díaz [3] P J Cardona [1 2 3 4]
1: Unitat de Tuberculosi Experimental, Microbiology Dept. Germans Trias i Pujol Research Institute and Hospital (IGTP-HUGTIP), Badalona, 08916, Spain 2: Genetics and Microbiology Department, Autonomous University of Barcelona, Barcelona, 08193, Spain 3: Centre de Medicina Comparativa i Bioimatge de Catalunya (CMCiB), Badalona, 08916, Spain 4: Centro de Investigación Biomédica en Red de Enfermedades Respiratorias (CIBERES), Madrid, 28029, Spain

Latent tuberculosis infection (LTBI) affects a quarter of the world population, with a 5-15% risk of progression to active tuberculosis (ATB). LTBI and ATB harbour dormant *Mycobacterium tuberculosis*, whose low metabolic activity implies the administration of complex treatments with low adherence, favouring antibiotic resistance acquisition. Therefore, the search for new therapeutic strategies against dormant bacilli is essential to control TB. *Drosophila melanogaster* is a suitable animal model to evaluate the therapeutic efficacy of a wide variety of compounds. This has been demonstrated with *Mycobacterium marinum* and *Mycobacterium abscessus* infections. However, there is a lack of a Mtb infection model in *D. melanogaster*. We hypothesize that, as *D. melanogaster* grows at 25ºC, it will keep Mtb infection in dormant status. Hence, we want to establish a model of LTBI in *D. melanogaster* for the characterization of the pathogenic mechanism of the infection and the evaluation of new therapeutic strategies. After setting up the equipment for working with *D. melanogaster* in Biological Safety Level 3 (NSB3) facilities, we performed Mtb infections with different doses. We monitored the survival daily and measured bacillary load at different times post-infection. Our results showed no significant difference in survival rates between the control and infected groups. Nevertheless, the infection is maintained over time. Although the model is still being optimized for use in NSB3 facilities and for the establishment of a suitable procedure to assess bacillary load within the host, it will constitute an extraordinary tool for the study of new therapeutic strategies against dormant Mtb.

OR14

Single cells RNA sequencing of peripheral blood mononuclear cells reveals hyperinflammatory monocytes in patients with *Mycobaterium abscessus* pulmonary disease

F Di Marco [1] S de Pretis [2] A Gramegna [3] F Saliu [1] F Giannese [2] F Blasi [3] D M Cirillo [1] N I Lorè [1]
1: San Raffaele Scientific Institute, Emerging Bacterial Pathogens Unit 2: San Raffaele Scientific Institute, Center for Omics Sciences 3: Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Respiratory Unit and Cystic Fibrosis Adult Center

People colonized by *M. abscessus* (MABSC) such as patients with cystic fibrosis (pwCF) are at risk of developing MABSC Pulmonary Disease (MABSC -PD), which can result in varying clinical outcomes. To improve the clinical management of MABS-PD, there is a need for reliable biomarkers such as immune signatures. In our study, we collected blood samples from adult pwCF and divided them into three groups based on their infection status and clinical stability: pwCF with no history of NTM detection and clinically stable (CF); pwCF NTM-colonized (MABSC); pwCF with clinical diagnosis for pulmonary disease (MABSC-PD). We then performed single cell RNA sequencing and Luminex assays on isolated PBMCs and plasma to identify transcriptomic/molecular signatures characterizing NTM-PD. We found alterations in several cell types, with the most interesting changes occurring in classical monocyte cells in MABSC-PD compared to CF or MABSC groups. Our analysis identified a unique RNA signature consisting of 47 upregulated and 24 downregulated genes. In plasma, CXCL10 levels were significantly higher in MABSC-PD than in other groups among 23 proinflammatory cytokines. Moreover, we validated our cellular target in a second public available datasets from a cohort of CF patients infected by *M. abscessus*. Our results suggest that hyperinflammatory monocyte responses are present in pwCF at risk of developing MABSC-PD.

Supported by the Italian CF Foundation FFC#23/2020 and FFC#7/2020

OR15

Cysteamine/Cystamine exert anti-*Mycobacterium abscessus* activity alone or in combination with amikacin.

I Palucci [1 2] A Salustri [1] F Di Maio [2] M DC Pereyra Boza [1] F Paglione [2] M Sali [1 2] L Occhigrossi [3] M D'Eletto [3] F Rossin [3] D Goletti [4] M Sanguinetti [1 2] M Piacentini [3 4] G Delogu [1 5]
1: Università Cattolica del Sacro Cuore 2: Fondazione Policlinico Universitario "A. Gemelli" 3: Università degli Studi di Roma "Tor Vergata 4: National Institute for Infectious Diseases Translational Research Unit, IRCCS 'Lazzaro Spallanzani' 5: Mater Olbia Hospital

Host-directed therapies are emerging as a promising tool in the curing of difficult-to-treat infections, such as those caused by drug-resistant bacteria. In this study, we aim to test the potential activity of the FDA- and EMA-approved drugs cysteamine and cystamine against *Mycobacterium abscessus*. In human macrophages (differentiated THP-1 cells), these drugs restricted *M. abscessus* growth similar to that achieved by amikacin. Here, we use the human ex vivo granuloma-like structures (GLS) model of infection with the *M. abscessus* rough (MAB-R) and smooth (MAB-S) variants to study the activity of new therapies against *M. abscessus*. We demonstrate that cysteamine and cystamine show a decrease in the number of total GLSs per well in the MAB-S and MAB-R infected human peripheral blood mononuclear cells (PBMCs). Furthermore, combined administration of cysteamine or cystamine with amikacin resulted in enhanced activity against the two *M. abscessus* morpho variants compared to treatment with amikacin only. Treatment with cysteamine and cystamine was more effective in reducing GLS size and bacterial load during MAB-S infection compared with MAB-R infection. Moreover, treatment with these two drugs drastically quenched the exuberant proinflammatory response triggered by the MAB-R variant. These findings showing the activity of cysteamine and cystamine against the R and S *M. abscessus* morphotypes support the use of these drugs as novel host-directed therapies against *M. abscessus* infections.

OR16

The spatial distribution of type 1 and type 17 immune transcriptomics profiles in murine models of chronic lung infection by opportunistic pathogens

F Nicola [1] F Di Marco [1 3] F Saliu [1] C Oneto [1] S De Pretis [1] F Giannese [1] D M Cirillo [1] N I Lorè [1 3]
1: San Raffaele Scientific Institute 2: Universita Vita-Salute San Raffaele 3: Fondazione Centro San Raffaele

The spatial distribution of local immune responses induced by chronic lung infections was not well characterized. Recently developed technologies allow to determine the local transcriptomic profile associated with inflamed tissues. Here, we exploited Visium spatial technology, and we aim at identifying the transcriptomic profiles and the tissue location of type 1 or type 17 immune

response in murine lungs during long-term chronic bacterial infection respectively by *Mycobacterium abscessus* (*Mab*) and *Pseudomonas aeruginosa* (*Pa*). Visium Spatial Gene Expression was performed from formalin-fixed paraffin-embedded (FFPE) lungs and ended with downstream analyses, such as spot deconvolution, results were validated by FACs/immunochemistry analysis. Lung tissue with chronic *Mab* infection was characterized by the presence of type 1 immunity and the formation of granuloma-inflated areas. Spatial inflammatory profiles of granuloma areas displayed a high local type 1 immune response in comparison to uninfected tissue, intended as a high proportion of immune cells and proinflammatory gene signatures related to Interferon-signalling and M1 macrophages phenotype. Similarly, the dynamic change in the location of type 17 immune response was observed in inducible bronchus-associated lymphoid tissue (iBalt) and close to the airways in comparison to inflamed areas in chronic *Pa* infection. These inflammatory profiles were validated by FACS and immunohistochemistry analysis in a second batch of mice. Our data show that spatial transcriptomics technology can be used in the field of host-pathogen interaction to study the spatial distribution of cellular transcriptomic profiles associated with granuloma structures.

OR17

Nanomotion technology in combination with machine learning: a new approach for rapid antibiotic susceptibility test for *Mycobacterium tuberculosis*

A Vocat [1 2] A Sturm [2] G Jozwiakb [2] G Jozwiakb [2] G Cathomen [2] M Swiatkowski [2] R Buga [2] G Wielgoszewski [2] D Cichocka [2] G Greub [1 3] O Opota [1]
1: Institute of Microbiology, Lausanne University Hospital and University of Lausanne, Lausanne, 1011, Switzerland 2: Resistell AG, Muttenz, 4132, Switzerland 3: Service of Infectious Diseases, Lausanne University Hospital and University of Lausanne, Lausanne, 1011, Switzerland

Backgrounds:

The antibiotic susceptibility of Mycobacterium tuberculosis (MTB) is assessed using both phenotypic and molecular methods. Phenotypic culture-based assays, are hindered by MTB's slow growth. Nanomotion technology is a growth-independent approach that records vibrations of bacteria attached to microcantilevers. Changes in the extent of vibrations upon antibiotic exposure can distinguish susceptible from resistant bacteria.

Methods:

We developed a nanomotion-based protocol for AST in Mycobacterium tuberculosis MTB, which was applied to predict strain susceptibility to isoniazid (INH) and rifampicin (RIF) using leave-one-out cross-validation (LOOCV) and machine learning techniques.

Results:

The MTB-nanomotion protocol takes 21 hours including preparation of the cell suspension, optimized bacterial attachment to functionalized cantilever and nanomotion recording upon antibiotic exposure. We applied this protocol combined LOOCV and machine learning to MTB isolates (n=40) and were able to discriminate between susceptible and resistance for INH and RIF with a maximum sensitivity of 97.4% and 100% respectively and the maximum specificity for both antibiotics was 100%; when considering each nanomotion recording independently. Triplicate experiments improved sensitivity and specificity to 100% for both antibiotics.

Conclusions:

Nanomotion technology has the potential to reduce the time required for MTB antibiotic susceptibility testing (AST) to just a few hours, compared to the days or weeks required for current phenotypic methods. This technology could also be expanded to test other anti-TB drugs, including newer drugs like bedaquiline, providing more effective guidance for TB treatment.

OR18

Successful *Myocbacterium tuberculosis* culture isolation from spiked tongue swabs processed by the Kudoh-Ogawa or cetylpyridinium chloride methods.

L Rigouts [2] R Reenaers [2] K Fissette [2] S Sengstake [2] B C de Jong [2]
1: BCCM/ITM 2: Institute of Tropical Medicine, Antwerp, Belgium 3: BCCM cooridination cell, Belgian Federal Science Policy, Brussels, Belgium

Tongue swabs are a promising sampling alternative to diagnose pulmonary tuberculosis (TB), especially in patients with difficulty to produce sputum. Molecular testing of tongue swabs showed encouraging sensitivity compared to sputum. Despite improvements in genotypic drug-susceptibility testing (DST), phenotypic DST remains the gold standard for many anti-TB drugs, requiring a cultured isolate. The elimination of commensal flora is critical to avoid culture contamination. In this study, we compared the performance of two simple decontamination methods on spiked tongue swabs taken from healthy volunteers.

The Kudoh-Ogawa (KO) method inactivates commensal flora using NaOH followed by inoculation directly on slightly acidic Ogawa, while decontamination by cetylpyridinium chloride (CPC) is followed by inoculation on neutral pH Löwenstein-Jensen (LJ). To evaluate whether *Mycobacterium tuberculosis* (Mtb) but not the commensal flora from tongue swabs can survive CPC/LJ and KO, we processed tongue swabs (Copan Floqswab) taken from 20 volunteers with both methods, versus control swabs without decontamination. Half of the samples were spiked with the H37Rv reference strain (10 µl of 10^{-4} dilution McFarland #1). Both methods reduced contamination significantly (85% to 0.5% for KO and 30% to 2.5% for CPC/LJ), while Mtb recovery was high (97%). Storage in CPC for less than 7 days did not impact Mtb growth, while recovery of H37Rv gradually decreased thereafter, with less abundant and slower growth.

Our results suggest that current sputum decontamination methods also work for tongue swabs. Work in progress assesses culture in liquid systems and evaluates swabs from pulmonary TB patients in field conditions.

OR19

Deeplex Myc/TB directly on sputum detects more mixed infections and heteroresistance compared to culture-based whole genome sequencing.

F Massou [1 2 3] P Supply [4] W Mulders [2] B C de Jong [2] D Affolabi [1] L Rigouts [2 3] C J Meehan [5]
1: Laboratoire de Reference des Mycobacteries 2: Institute of Tropical Medicine 3: University of Antwerp 4: Genoscreen 5: Nottingham Trent University

Whole genome sequencing (WGS) analysis of *M. tuberculosis* (MTB) still relies on culture, which may introduce bias by losing fastidious growers and/or minority variants. Direct sequencing on sputum may provide more clinically and epidemiologically relevant information.

In the DIAMA study comprising 9 sub-Saharan African countries, we enrolled equal numbers of Xpert positive patients with rifampicin resistant (RR)-TB and retreatment patients with RS-TB. We compared the performance of Deeplex Myc/TB (Genoscreen, France) directly on sputum with WGS from respective positive cultures, to detect the presence of heteroresistance (resistance mutations < 90%) and mixed infections. Analysis was done using an integrated platform (Deeplex) and TB profiler Vs4.4.2 (WGS).

Among a total of 2132 samples, 1814 yielded valid results by Deeplex and 1131 by culture followed by WGS (cWGS). Multiple lineages were detected in 10% of specimens by Deeplex versus 1% of isolates by cWGS .This difference was primarily driven by Lineages 5 and 6 not being detected in mixed infections by Cwgs (OR, p< 0.01). Single clone heteroresistant sub-populations were detected in 9% by Deeplex and 6% by cWGS.

These findings suggest that culture-based WGS misses large proportions of mixed infections and heteroresistance, likely due to inherent biases selecting specific strains during the culturing process. Strains missed by cWGS were primarily L5 and L6, which has large implications for cWGS-based epidemiological studies in West Africa. Further work is required to develop direct from clinical sample WGS to overcome these biases in future studies.

OR20

Diagnostic accuracy of upper airway swabs and saliva with Xpert MTB/RIF Ultra for the detection of tuberculosis in adults

M de Vos [9] N van Hung [1] C A Ugarte-Gil [2 3] H Cox [4] K Ali [2] N Hapeela [4] M Joloba [5] R Post [6] S Kim [6] S Kennedy [8] M Ruhwald [9] J Ellnor [7] A Penn-Nicholson [9] S Dorman [8]
1: National Tuberculosis Reference Laboratory, National Tuberculosis Control Programme, National Lung Hospital, Hanoi, Viet Nam 2: Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, 15102, Peru 3: School of Medicine, Universidad Peruana Cayetona Heredia, Lima, 15102, Peru 4: Division of Medical Microbiology, Department of Pathology, University of Cape Town, Cape Town, 7925, South Africa 5: Department of Microbiology, School of Biomedical Sciences, Makerere University, Kampala, 7062 Uganda 6: Department of Biostatistics, Frontier Science Foundation, Madison, WI 53711 and Brookline, MA 02446, USA 7: Center for Emerging Pathogens, Department of Medicine, Rutgers-New Jersey Medical School, Newark, NJ, USA. Newark, 07103, USA 8: Department of Medicine, Medical University of South Carolina, Charleston, 29425, USA 9: FIND, Geneva, 1202, Switzerland

To regain progress lost during the COVID-19 pandemic and reduce the global burden of tuberculosis (TB), diagnostic innovations are critically needed. Upper airway swabs offer a promising alternative sample type to sputum as collection is rapid, non-invasive and can be obtained from non-sputum productive patients.

As part of the FEND-TB multicentre clinical study (adults, symptomatic for TB), we assessed the diagnostic accuracy of Xpert MTB/RIF Ultra (Ultra) for the detection of pulmonary TB using saliva and swabs sampled from four different upper airway sites. Swabs were collected in 1.2mL PBS buffer and Ultra testing was done using the manufacturer's instructions. Using the composite of sputum culture and/or Ultra as the microbiological reference standard (MRS), 60 sputum smear-positive and 30 smear-negative MRS-positive, and 30 MRS-negative participant samples were evaluated. Upper airway swabs from smear-positive participants had Ultra sensitivity of 88% (95% CI 80-95), 67% (95% CI 50-79), 74% (95% CI 57-85) and 55% (95% CI 38-72) from tongue dorsum, cheeks and gums, combined tongue-cheeks-gums, and mid-turbinate nasal swabs. Specificity was 100% for all swab types. In smear-negative, sensitivity was 16.7% (95% CI, 7.3-33.6) from the tongue dorsum. In saliva, sensitivity was 100% (95%CI 87.5-100) in smear-positive and similarly poor in smear-negative cases (17.6% (95% CI, 7.7-35.4)).

The poor performance of oral swabs from smear-negative participants may be due to processing where only 55% of the total sample was tested. We will further discuss optimization of the Ultra workflow, determine the stability of tongue swabs and feasibility of testing serially collected swabs.

OR21

The simple one-step stool processing method to diagnose tuberculosis is robust enough for global scale up

P de Haas [1] B Yenew [2] A Slyzkyi [1] K Kremer [1] E Klinkenberg [3] E Tiemersma [1]
1: KNCV Tuberculosis Foundation 2: Ethiopian Public Health Institute 3: Independent consultant, Connect TB

Introduction
The diagnosis of pulmonary tuberculosis (TB) in children and people living with HIV is hampered because of nonspecific symptoms and lack of laboratory confirmation due to paucibacillary load and difficulty to obtain sputum. Use of stool as an alternative non-invasive sample is recommended by the World Health Organization as an initial diagnostic test for the detection of TB and rifampicin resistance in children with signs and symptoms of pulmonary TB.

Methods
The simple one step stool processing (SOS) method and Xpert-Ultra testing were used to detect *Mycobacterium tuberculosis* and rifampicin resistance. This method uses the same supplies and equipment as used for sputum testing. Several countries have introduced this method at program level. We have investigated the robustness of the SOS method by varying the quantity of stool, storage temperature, sedimentation time, shaking method and transport conditions.

Results
In total 2,963 Xpert-Ultra results from 132 stool specimens of 47 TB patients were obtained. We compared Xpert-Ultra processing errors and MTB-positivity rates between the standard and modified stool processing procedures. Minor deviations from the standard SOS method did not significantly impact the Xpert-Ultra test results.

Conclusion
We confirmed the robustness of the SOS stool processing method and Xpert-Ultra testing for the detection of TB. The results showed that a wider range of stool quantity (0.3-0.8g) than we advised previously (0.8g) can be used without compromising Xpert test results. The SOS method provides a simple and cost-effective work-up close to point of care that is sufficiently robust for global scale-up.

OR22

Evaluating DNA extraction commercial kits from *Mycobacterium tuberculosis* clinical primary liquid (MGIT) culture for downstream sequencing applications

E C Conceicao [1] M Ismail [1] F Wells [1] B Mann [1] A Paulse [1] I Al-Talib [1] J Williams [1] A Dippennaar [1 2] G Van der Spuy [1] A Van Rie [2] R M Warren [1]
1: South African Medical Research Council Centre for Tuberculosis Research, Division of Molecular Biology and Human Genetics, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa 2: Department of Family Medicine and Population Health, Global Health Institute, Faculty of Medicine and Health Sciences, University of Antwerp, Antwerp, Belgium

Whole-genome sequencing (WGS) has great potential for determining the complete drug-resistance profile of clinical *Mycobacterium tuberculosis* (*Mtb*) strains. Cetyltrimethylammonium-bromide (CTAB), the reference method for *Mtb* DNA extraction, is labour intensive and difficult to implement in routine laboratory settings. We evaluated the performance of commercial kits to extract DNA from clinical primary liquid cultures (CLPC). *Mtb*-positive decontaminated sputum sediments were pooled and used to inoculate mycobacteria-growth-indicator-tube (MGIT) 960 media. Positive non-contaminated MGIT cultures were pooled to generate 10 replicate isolates for DNA extraction by CTAB method with (+) or without (-) RNAse and seven commercial kits (+/- modifications): Zymo-DNA Clean and Concentrator, Zymo-Quick-DNA Fungal/Bacterial (+lysozyme-digestion), InstaGene (+/-bead-beat), GenoLyse (+/-precipitation), FluoroLyse (+/-precipitation), PrepGEM-Bacterial (+/-precipitation), NucleoSpin-Tissue, Nucleomag-Pathogen, and Gene-Xpert buffer (+precipitation or +purification). Genomic libraries for WGS were generated using Illumina DNA-Prep Kit and quality controlled using Qubit-dsDNA/HS and TapeStation. Nine performance parameters were evaluated: quantity of total dsDNA, DNA purity (Nanodrop spectrophotometry), DNA integrity through agarose-gel electrophoresis and PCR targeting the *pncA* gene (615 bp), quantity of genomic libraries (ng/uL), library fragment size (bp), turnaround time and cost. We developed a score for each performance parameter (1 to 5), used a radar-plot to visualize the scores and calculated the sum of scores for each method. The three best-performing methods were InstaGene +/-bead-beat (score=35), CTAB +RNAse (score=34), FluoroLyse +precipitation (score=33). The poorest performing kit was Zymo-Quick-DNA Fungal/Bacterial (score=11). Further evaluation by integrating WGS data quality scores will be performed to select the optimal commercially available kit(s) for routine downstream sequencing applications on CLPCs.

OR23

The role of MIC shifts as early markers of treatment failure in tuberculosis

M Moreno-Molina [1] C Mariner-Llicer [1] B Saavedra [2 3] E Mambuque [2] N Gomes [2] M Torres-Puente [1] L Villamayor [4] P Cano-Jimenez [1] M G Lopez [1] A Garcia-Basteiro [2 3] I Comas [1 5]
1: Biomedicine Institute of Valencia IBV-CSIC 2: Centro de Investigação em Saúde de Manhiça 3: ISGlobal, Barcelona Centre for International Health Research, Hospital Clínic - Universitat de Barcelona 4: FISABIO Public Health 5: CIBER in Epidemiology and Public Health

Understanding the pathogen genetic basis of treatment failure beyond canonical resistance in tuberculosis (TB) can have a major impact on its global control. In this work we performed deep whole-genome sequencing in serial TB samples from 40 patients from Mozambique during their first month of treatment. Then, using the EUCAST protocol, we determined the minimum inhibitory concentration (MIC) of six antitubercular drugs -isoniazid, rifampicin, ethambutol, streptomycin, levofloxacin and amikacin- for the baseline and a follow-up sample of every patient. We sought to assess how antibiotic selective forces shape tuberculosis population dynamics and diversity during the first weeks of treatment, and evaluate the impact of mutations both inside and outside of the canonical resistance genes in the overall MICs.

Analyzing the population dynamics between both samples, we detected that 26 patients experienced a decreased capacity to eliminate TB diversity during this early stage of treatment, and we correlated this finding with longer periods of culture positivity. Concurrently, we identified 12 cases where the MIC shifted to higher drug concentrations during treatment, with 10/12 failing to reduce diversity or become culture-negative. Thanks to deep sequencing, we could observe transient non-canonical heteroresistance at low frequencies, providing candidates to explain some of these MIC shifts. We reason that subtle shifts in minimum inhibitory concentration during early treatment may serve as a clinical tool to predict a reduced capacity to eliminate bacterial diversity but will need further validation. This, in turn, could be an indicator of worse prognosis and eventual treatment failure.

OR24

Ph neutral anti-microbial peptide-based decontamination of samples enhances recovery in culture of low load *Mycobacterium tuberculosis*

T J Bull [1] T Munshi [1] C Cosgrove [1] A Bartolf [2] M Meninchini [2] L Rindi [2] S Esin [2] G Batoni [2] K Hilpert
1: St. George's University of London 2: University of Pisa

Culture confirmation of *M.tuberculosis* (MTB) remains the gold standard for diagnosis of Tuberculosis but is typically successful in only 60% of samples even though many patients remain infectious.

We evaluated replacing the NaCl decontamination (gold standard) protocol of samples, followed by MGIT culture, with a Ph neutral pan anti-microbial peptide-based sample decontamination procedure followed by MGIT culture supplemented with a D-enantiomer peptide MTB growth enhancer (Index test).

255 clinical specimens obtained from 145 patients with a possible or diagnosed Tuberculosis were split evenly and processed in parallel. The Index test was significantly (P<0.0001) superior to the gold standard, recovering 56 MTB positive cultures including 13 faeces vs 30 MTB positive cultures including 1 faeces. It increased the culture confirmation rate for diagnosed Tuberculosis patients by 10% and empirically treated patients by 14%. The Index method also decreased sample contamination rate by 12%, increased the sample positivity rate by 46%, sampling test sensitivity by 19%, and provided a median decrease of 7.5 days in time to MGIT culture positivity (Ratio 0.6809 : 95% CI 0.44 to 1.05).

We conclude that D-enantiomer antimicrobial peptide-based decontamination and culture provides increased sensitivity, rapidity and recovery of MTB, particularly from low load clinical samples, offering potential for improvement of culture confirmation during smear negative respiratory and faecal sampling.

OR25

Detection of Mycobacterium tuberculosis and resistance mutations in different sample types using FluoroType MTBDR v2. A study from Germany and Denmark.

E Svensson [1] H Ketelsen [2] D Hillemann [2]
1: International Reference Laboratory of Mycobacteriology, Statens Serum Institut, Copenhagen 2: National and WHO Supranational Reference Laboratory for Mycobacteria, Research Centre Borstel, Borstel

Objective: The FluoroType MTBDR version 2 (FTv2) is a qualitative real-time PCR for simultaneous detection of *Mycobacterium tuberculosis* complex (MTBC) DNA and mutations conferring resistance to rifampicin (RIF) and isoniazid (INH). We evaluated the performance of the FTv2 assay to detect MTBC in respiratory and extrapulmonary samples in a low tuberculosis prevalence setting. In addition, we investigated the performance of the FTv2 assay to detect resistance to RIF or INH in primary samples.

Methods: 1815 fresh samples from Denmark and 445 frozen samples from Germany were tested by the FTv2 assay and compared to culture. Results were compared to phenotypic antimicrobial susceptibility testing and a composite reference DNA (CRD) comprising the HAIN MTBDR line probe assay and sequencing or whole genome sequencing. In addition, to the ability of the FTv2 assay to detect RIF and INH resistance mutations, 110 frozen samples from Sierra Leone were analysed.

Results: In detecting MTBC in sputum, the sensitivity for German samples was 0.90 (CI 0.86-0.94), and specificity was 1.00 (0.89-1.00). The corresponding values were 0.86 for Denmark (0.71-0.94) and 0.98 (0.97-0.99). As for detecting resistance mutations, the sensitivity was 0.99 (0.95-1.00) for RIF and specificity 0.96 (0.97-1.00), while for INH mutations, they were 1.00 (0.96-1.00) and 0.98 (0.95-0.99).

Conclusion: FTv2 is a reliable tool for detecting MTBC DNA in pulmonary and extrapulmonary samples and detecting high-confidence mutations for INH and RIF resistance in inhA promoter, katG, and rpoB.

OR26

Consideration of the results of the Xpert MTB/RIF method on the treatment outcome of patients with M\XDR pulmonary tuberculosis during the COVID-19 epidemic in Kharkiv region, Ukraine

TS Butova(1) OS Solodiankin(2,3) N Rudova(2) NM Sapelnik(3) OL Borysova(3) DO Butov(3)
1: Research Institute of Experimental and Clinical Medicine, Kharkiv National Medical University, Kharkiv, 61022, Ukraine; 2: Laboratory of Molecular Diagnostics, National Scientific Center Institute of Experimental and Clinical Veterinary Medicine, Kharkiv, 61023, Ukraine; 3: Department of Phthisiology and Pulmonology, Kharkiv National Medical University, Kharkiv, 61022, Ukraine

The **aim** of our study was to determine the presence or absence of the effect of the Xpert MTB/RIF method on the treatment outcome of patients with multi\extensively drug-resistant tuberculosis (M\XDR-TB) of the lungs during the COVID-19 epidemic in Kharkiv region, Ukraine.

Methods. A prospective observational cohort study was performed on registry data from the regional antituberculosis dispensary #1 in Kharkiv region, Ukraine. All microbiologically confirmed M\XDR-TB patients registered in 2020 were included. All samples of the culture of *Mycobacterium tuberculosis* of patients were subjected to whole genome sequencing. Diagnostic and treatment data were analyzed. The treatment outcomes of tuberculosis (TB) were assessed according to WHO recommendations for the period of the study. One of the exclusion criteria was “Lost to follow up” as an outcome of TB treatment.

Results. We observed 168 patients with M\XDR-TB. Patients were divided into two groups: group 1 - patients who did not receive Xpert MTB/RIF upon admission to the dispensary (n=44) and group 2 - received Xpert MTB/RIF (n=124). Treatment failure and death were observed in 26 (59.1%) patients in group 1 and 45 (36.3%) patients in group 2, p=0.0313. 18 (40.9 %) patients recovered and completed treatment in group 1 and 79 (63.7 %) patients in group 2, p=0.0313 against the background of treatment duration (mean (±SD)): group 1 – 611.8 ±43.1 and group 2 - 567.2±105.9 days, p=0.0001.

Conclusions. Pre-Treatment Results Xpert MTB/RIF in the early days of hospital admission provides better efficacy and shorter duration of chemotherapy for patients with M/XDR-TB.

OR27

Exploring the plasmidome of non-tuberculous mycobacteria

M Diricks [1] N Wetzstein [2] M Merker [1] S Niemann [1]
1: Research center Borstel 2: University Hospital Frankfurt

Non-tuberculous mycobacteria (NTM) are ubiquitous in the environment and some species are causing rising numbers of infections in humans. Yet, it is unclear if this is due to an increase in virulence of some NTM strains or due to other factors. Plasmids for instance may play a pivotal role in transmitting virulence factors and resistance genes. However, studies focusing on these genetic elements in NTMs are scarce. Here, we determine the prevalence, characteristics and diversity of plasmids using different plasmid databases (NCBI and PLSDB). In addition, we evaluated the ability of different state-of-the-art plasmid prediction tools (plasmidspades, platon and SRST2) to reconstruct plasmids from short read sequencing data. A total of 199 complete genomes were available in NCBI for 18 clinically relevant NTM species. Among those, 60 genomes from nine species (including *M. abscessus*, *M. avium*, *M. intracellulare*, *M. kansasii*, *M. fortuitum*, *M. lentiflavum*, *M. marinum*, *M. ulcerans* and *M. gordonae*) harbored one up to five plasmids. The 154 NTM plasmids available in PLSDB were highly diverse with regard to length (4 kbp - 864 kbp), circularity (15% linear) and genomic content (few shared genes) but known resistance and virulence genes were typically absent. Genetically closely related plasmids were found in different NTMs, indicating that those plasmids can move across species barriers. Short sequencing reads can be used to detect the presence of known plasmids but *de novo* plasmid assembly remains a challenge. Overall, the results significantly broaden our knowledge of plasmids in NTMs and might facilitate further plasmidome studies.

OR28

Genomic landscape of *M. avium* complex in Central Germany

N Wetzstein [1 2] M Diricks [2 3 4] T A Kohl [2 3 4] S Andres [3 4] M Kuhns [3 4] I Friesen [3 4] S Niemann [2 3 4] T A Wichelhaus [5]
1: Department of Internal Medicine, Infectious Diseases, University Hospital Frankfurt, Goethe University, Frankfurt am Main, Germany 2: Molecular and Experimental Mycobacteriology, Research Center Borstel, Borstel, Germany 3: German Center for Infection Research (DZIF), partner site Hamburg-Lübeck-Borstel-Riems, Germany 4: National and WHO Supranational Reference Laboratory for Mycobacteria, Research Center Borstel, Leibniz Lung Center, Borstel, Germany 5: Institute of Medical Microbiology and Infection Control, University Hospital Frankfurt, Goethe University, Frankfurt am Main, Germany

The *Mycobacterium avium* complex (MAC) comprises the most frequent non-tuberculous mycobacteria (NTM) in Central Europe and currently includes twelve species. Clinically, the most relevant ones are *M. avium*, *M. intracellulare* subsp. *intracellulare* (MINT) and *M. intracellulare* subsp. *chimaera* (MCH) causing pulmonary or disseminated infections. However, the population structure and genomic landscape of MAC remain little investigated. To address this, Illumina sequencing was performed on 328 MAC isolates from 185 German patients

collected between 2006 and 2021 and set into context with publicly available MAC reference strains, as well as clinical data. Species were identified with *NTMprofiler* and sequences mapped to the according reference genomes *M. avium* ATCC 25291 or *M. intracellulare* ATCC 13950 with *MTBseq*. Overall, 229 isolates could be assigned to *M. avium*, 95 to *M. intracellulare* (46 MINT, 49 MCH), and two to *M. marseillense*. Although serial isolates showed high intrapersonal genetic stability, reinfection appeared to be a frequent event with 15/43 patients exhibiting genetically distinct MAC isolates (or even species) over the course of time. On the other hand, we identified clusters with less than 25 SNPs distance between genomes of isolates recovered from different patients, indicating possible transmission events both in *M. avium* and *M. intracellulare*. Interestingly, these clusters consisted of patients with a variety of underlying dispositions and clinical manifestations. In conclusion, this first comprehensive genomic study of MAC in a German cohort demonstrates that reinfection seems to be a frequent event, while clusters indicate possible person-to-person transmission or a common environmental source.

OR29

Frozen in Time: Unlocking the History of Nontuberculous Mycobacteria

X ES Iversen [1] A Norman [1] D B Folkvardsen [1] E Svensson [1] E M Rasmussen [1] T Lillebaek [1 2]
1: International Reference Laboratory of Mycobacteriology, Statens Serum Institut, Copenhagen, Denmark 2: Global Health Section, Department of Public Health, University of Copenhagen, Denmark

The global incidence and prevalence of nontuberculous mycobacteria (NTM) disease and colonization continue to rise. In a pilot study, we explored our unique collection of freeze-dried mycobacterial strains stored at the International Reference Laboratory of Mycobacteriology (IRLM) at Statens Serum Institut (SSI), Copenhagen, since 1948. We selected 28 'historical' NTM isolates collected from patients between 1948 to 1957. We investigated their viability using whole genome sequencing (WGS) on DNA extracted directly from a suspension of freeze-dried cells versus after culturing. The DNA quality was evaluated by analyzing the per-base quality scores of paired-end sequencing reads and the overall contiguity of resulting *de novo* assemblies. Remarkably, all isolates had remained viable after seven decades in storage, as they were easily re-cultured. No DNA degradation was observed when analyzing sequence data from the freeze-dried cells. These findings emphasize the value of freeze-drying for long-term storage and demonstrate that sequencing directly on freeze-dried mycobacteria without prior re-cultivation can save laboratory time and resources. Furthermore, analysis of historical isolates revealed four previously unknown *Mycobacterium* strains isolated from patients between 1948 to 1955. NTM infection and disease remain poorly studied and many aspects are still unknown, a field that needs to be improved. Our study lays the groundwork for future investigations of the IRLM strain collection, which comprises approximately 4,000 isolates. Furthermore, ongoing studies aim to enhance our understanding of NTM by exploring the course of epidemiology, etiology, and resistance mechanisms of NTM over decades.

OR30

Rifampicin substituted by clofazimine in the recommended therapy of *Mycobacterium avium* pulmonary disease: a hollow-fibre model study

S Salillas [1] J Raaijmakers [2] J van Ingen [2]
1: Universidad de Zaragoza 2: Radboud University Medical Center

The treatment of *M. avium* pulmonary disease consists of a long-term multidrug regimen (macrolide, ethambutol and rifampicin) that shows limited efficacy. Here, we assess the efficacy of clofazimine as a rifampicin substitute within the recommended treatment of *M. avium* pulmonary disease in an intracellular hollow-fibre infection model (HFIM). THP-1 cells were infected with *M. avium* ATCC 700898 and exposed to either a regimen of azithromycin, ethambutol rifampicin or azithromycin, ethambutol clofazimine for 21 days. Lung pharmacokinetic profiles (C_{max}, T_{max}, $T_{1/2}$) of azithromycin, ethambutol and rifampicin were simulated. For clofazimine, a C_{avg} was targeted. PK profiles were generated on the first day on day 16. Bacterial densities (azithromycin sensitive and resistant fraction) were determined at days 0, 3, 7, 14, and 21. On day 0 the C_{max} of azithromycin, ethambutol and rifampicin were lower than targeted whereas the clofazimine C_{max} was higher than targeted. Not all results are obtained up to day 21 yet. Until day 14 both rifampicin- and clofazimine-containing arms were able to maintain stasis. In the first week, rifampicin systems developed azithromycin resistance on day 3, whereas clofazimine ones did not show emergence of resistance. The substitution of rifampicin by clofazimine showed similar efficacy in terms of bacterial densities in the HFIM until day 14, although in the clofazimine arm there was no resistance observed at least until day 7. Therefore, substituting rifampicin with clofazimine might be a good alternative for the treatment of *M. avium* pulmonary disease.

OR31

Fine-scale evolution of *Mycobacterium tuberculosis* growth rate

K M Malone [1 2] L Lima [1] J Knaggs [1 2] M Hunt [1 2] P Fowler [2] D Crooke [2] Z Iqbal [1]
1: EMBL-EBI 2: University of Oxford

Understanding how modest genomic diversity amongst the *Mycobacterium tuberculosis* lineages influences fitness is vital for devising successful tuberculosis control strategies. Growth rate is intimately linked to fitness and to date, only a handful of studies have identified variation in growth rate between particular lineages *in vitro*. Here, we describe the evolution of growth rate across a phylogeny of over 10,000 clinical isolates representing major lineages 1-4 from 23 different countries. We find that the entire phylogeny can be divided into 11 different clades based on growth rate. Ancestral sequence reconstruction highlights genetic changes associated with growth rate that have become fixed in the emergence of specific sub-lineages prior to the introduction of antibiotics. Alteration to growth rate appears to have evolved independently amongst the clades with differences in metabolic pathways having a more significant impact on growth than convergent evolution in a specific set of genes. Finally, we demonstrate the usefulness of our clade classification for exploration of additional phenotypes by showcasing the

impact of variation at codon 306 of *embB* on the minimum inhibitory concentration of ethambutol and how genetic prediction of resistance to this drug should take account of sub-lineage.

OR32

CRISPR-Cas molecular memory in *Mycobacterium canettii* reveals the putative ancestral environmental origin of *M. tuberculosis*

A Ghodousi [1] M Marceau [2] C Gaudin [2] A Cabibbe [1] X Didelot [3] D Cirillo [1] P Supply [2]
1: Vita-Salute San Raffaele University, IRCCS San Raffaele Scientific Institute, Milan, Italy 2: Univ. Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, U1019 - UMR 9017 - CIIL - Center for Infection and Immunity of Lille, Lille, France 3: School of Life Sciences and Department of Statistics, University of Warwick, United Kingdom

Although it is often thought that the progenitor of the *Mycobacterium tuberculosis* complex (MTBC) arose from soil, its environmental origin remains elusive. In order to search for this ancestral source, we exploited unique combined features of CRISPR-Cas systems and *M. canettii* TB clinical strains. As prokaryotic CRISPR-Cas systems provide adaptive immunity to foreign invasion by storing specific spacer sequences excised from viral genomes and other parasitic genetic elements, CRISPR spacers represent a molecular memory of prior microbial ecology. In contrast with the MTBC, *M. canettii* strains are recombinogenic, show diverse CRISPR-Cas systems, and represent evolutionarily early branching lineages of tubercle bacilli, with an unknown putative environmental reservoir in East Africa. Therefore, we identified and comprehensively analyzed CRISPR-cas loci and spacer repertoires in all previously known and newly sequenced genomes of *M. canettii*, and in representative genomes of all known human- and animal-associated lineages. On top of CRISPR-Cas Type III-A (shared with the MTBC), I-C and I-E previously known, Type I-D and I-U systems were newly identified. Among multiple other events of horizontal evolution among *M. canettii* strains, phylogenetic reconstruction and CRISPR-cas structural analysis indicated the involvement of an environmental mycobacterium-like ancestor in past horizontal exchanges of the CRISPR-cas locus from a particular strain subgroup. Moreover, we found multiple CRISPR spacers in *M. canettii* and some MTBC spacers that match sequences of environmental mycobacteriophages or prophages integrated in genomes of free-living (myco)bacteria. The distribution of the environmental sources of these organisms indicates a non-telluric ancestral origin for the MTBC

OR33

Paleopathological and molecular evidence of tuberculosis in human skeletal remains from 18th-19th century Orthodox cemeteries in Irkutsk, Eastern Siberia

I Mokrousov [1] N Kharlamova [2] I Berdnikov [3] N Berdnikova [3] R Galeev [2] O Ogarkov [4]
1: St. Petersburg Pasteur Institute, St. Petersburg, Russia 2: N.N. Mikloukho-Maklay Institute of Ethnology and Anthropology, Russian Academy of Sciences, Moscow, Russia 3: Scientific Research Center Baikal Region, Irkutsk State University, Russia 4: Scientific Centre of the Family Health and Human Reproduction Problems, Irkutsk, Russia

The city of Irkutsk in Eastern Siberia was founded in the 17th century by settlers from Russia. Compared to the large cities of European Russia, its population was small (from 1,000 to 10,000 during 18th century), but the overcrowding-related conditions favorable for the spread of transmissible pathogens were still present. Here, we tested the skeletal human remains from the 18th-19th centuries' Orthodox cemeteries of the Spasskaya and Krestovozdvizhenskaya Churches in Irkutsk for tuberculosis-associated bone alterations and *Mycobacterium tuberculosis* DNA. The morphologically examined skeletal collection included 591 individuals of Caucasian origin. Among infectious lesions, those characteristic of osteomyelitis, syphilis, and tuberculosis were found. The molecular analysis (PCR, real-time PCR of IS*6110* and other markers, and spoligotyping) concordantly suggested that at least four individuals (out of 15 TB-suspected, DNA-tested) were positive for the presence of *M. tuberculosis* DNA. They were all males (3 maturus and 1 maturus senilis age) dated between the 1730s and 1810s. The combined molecular analysis suggested a presence of different strains. At least some of them appear to represent not the currently predominant in Siberia the Beijing genotype (*M. tuberculosis* East-Asian lineage) but strains of the European descent. In conclusion, this study presented bioarchaeological and molecular evidence of tuberculosis in human skeletal remains from 18th-19th centuries Orthodox cemeteries in Irkutsk, Siberia. The samples are not *M. bovis* and represent human *M. tuberculosis sensu stricto*. Their precise phylogenetic identity is elusive but cautiously evokes the European/Russian ancestry of at least some isolates.

Funding: Russian Science Foundation (grant 19-14-00013).

OR34

From bad to worse: Does zinc limitation make *M. tuberculosis* more virulent?

S Prisic [1]
1: University of Hawaii at Manoa

Mycobacterium tuberculosis (Mtb) residing within the core of necrotic granulomas are difficult to eradicate. These persisters are not well-characterized, thus hindering discovery of new TB treatments. As a part of the nutritional immunity, zinc ion (Zn^{2+}) is sequestered in necrotic granulomas. We hypothesize that Zn^{2+} limitation in necrotic granulomas leads to formation of a distinct Mtb subpopulation, which alters their interaction with the host and allows survival. We previously demonstrated that Zn^{2+} limitation causes physiological changes in Mtb that go well

beyond Zn^{2+} conservation, but likely prepare Mtb for impending phagocytosis. Compared to Zn^{2+}-replete bacteria, Zn^{2+}-limited Mtb have altered cell wall, higher tolerance to oxidative stress, different susceptibility to certain antibiotics, and increased virulence in the mouse infection model. Our current study aims to understand how this adaptation influences Mtb's interactions with the host. Therefore, we investigated how macrophages respond to these two different Mtb subpopulations using transcriptomics, cell viability assays, reactive oxygen species (ROS) production, and bacterial uptake and survival analysis. The results of this study indicate that macrophages are able to distinguish between the two bacterial subpopulations, i.e., Zn^{2+}-replete and Zn^{2+}-limited Mtb, and the latter results in increased bacterial uptake, more macrophage death, decreased pro-inflammatory response, and higher ROS production. Therefore, by attempting to limit Mtb growth, the host defense creates even more adversarial enemy. Considering that most studies have been conducted with Zn^{2+}-replete Mtb, the newly discovered unique characteristics of the Zn^{2+}-limited Mtb and their distinct interactions with macrophages may be the key for improving TB therapy.

OR35

Evolution of drug resistance and treatment outcome within a longitudinal retrospective study – linking outcome to treatment.

M Grobbelaar [1] R M Warren [1] H Cox [2] A Dippenaar [3]
1: Stellenbosch University 2: University of Cape Town 3: University of Antwerp

We investigated 47 DR-TB cases with multiple isolates in a large retrospective cohort study in a high TB-incidence setting in Cape Town, South Africa. DNA was extracted using the CTAB extraction method. WGS was performed using the Illumina platform. Sequences were analyzed with a customized pipeline using open-source software. Drug resistance-conferring and associated mutations were identified using TB-Profiler.

The cohort consisted of 18 female and 29 male patients. WGS data were used to divide the cohort into groups, 29 patients had changes in their drug-resistance (DR) profiles while 18 patients had the same DR profile irrespective of episodes. Of the 18 patients (same DR), 16% had no previous TB while 83% had previous TB. In the group with DR changes, 13% had no previous TB, while 86% had previous TB, indicating that previous TB did not influence the evolution of DR within the new episodes. When treatment was linked to the outcome, 64% of episodes were cured when patients received ≥ 4 drugs however 31% of patients were still cured even with < 3 effective drugs. More patients also failed treatment when they received < 3 effective drugs (50%), however, 21% of patients still failed regardless of receiving ≥ 4 drugs. Of patients with an LTFU outcome, 52% received 3 drugs, while only 33% of patients received ≥ 4 effective drugs. Finally, more patients died when they received ≥ 4 drugs compared to 7% and 29% that received 3 or ≤ 3 effective drugs respectively.

OR36

Next-generation sequencing cluster typing; All single nucleotide polymorphisms are equal but some are more equal than others

R de Zwaan [1] E Ubbelohde [1 2] A Mulder [1] M Kamst [1] G de Vries [1] K Rebel [3]
S NS Kautz [4] D van Soolingen [1] K Kremer [2] R M Anthony [1]
1: National Institute for Public Health and the Environment 2: KNCV Tuberculosis Foundation
3: Municipal Public Health Services, Amsterdam 4: Municipal Public Health Services, Rotterdam

In the Netherlands both molecular typing and epidemiological data relating to transmission of tuberculosis (TB) is investigated. In this study we investigated a large Dutch TB cluster of more than 150 isolates over 25 years. Next-generation sequencing (NGS) data from 61 isolates (2003-2022) was available including one follow-up isolate and two cases of reactivation**.** Our routine NGS pipeline calls isolates as potentially epidemiologically linked on the basis of a standard 12-single nucleotide polymorphism (SNP) distance cutoff. Identifying additional information in the NGS data could guide epidemiological investigations and improve our understanding of transmission chains. We supplemented our automated analysis with a more detailed manual investigation of cluster "specific" SNPs and evaluated their confidence and the presence of minority SNP populations or unfixed SNPs. The SNP analysis was consistent with the epidemiological data and identified two nonsynonymous *dnaA* mutation (M348I, R400H) events that transitioned from an unfixed SNP to a fixed SNP. Mutations in *dnaA* have previously been observed by others as emerging among clustered isolates and are associated with reduced *katG* expression, potentially improving mycobacterial survival during isoniazid treatment. Thought most of the high confidence SNPs acquired within this cluster were unique in our Dutch database (>4,000 TB isolates) one of the high confidence mutations in the *dnaA* gene was present in eight unrelated isolates. The examination of high confidence SNPs and the presence of fixed and unfixed SNPs detected both inter- and intrapatient microevolution within this cluster, and provided additional resolution for the identification of epidemiologically linked isolates.

OR37

Improving vaccine descriptions in model-based impact prognosis of new tuberculosis vaccines: removing arbitrariness and reducing bias.

J Sanz [2 4] M Tovar [2 4] Y Moreno [2 3 4]
1: Universidad de Zaragoza 2: Universidad de Zaragoza - Instituto BIFI 3: ISI Foundation
4: Universidad de Zaragoza - Dept. Física Teórica

The development of vaccines against tuberculosis (TB) poses a series of unique challenges when compared to other diseases. Among these, evaluating their efficacy through randomized control trials (RCTs), and mapping it to prospective impact forecasts based on mathematical modeling is one of paramount complexity. Furthermore, it is a task of utmost importance, given the scarcity of resources for vaccine development in the fight against TB.

One reason underlying this difficulty stems from the co-existence of different routes to disease in the natural history of the disease, (primary TB, endogenous reactivation from latent infection, and TB upon exogenous re-infection). This fact makes it challenging to translate RCT-derived efficacy estimates into specific mechanistic interpretations of vaccine behavior needed to inform mathematical models. This problem is especially relevant when comparing impact forecasts for vaccines with different product profile characteristics, and/or tested through RCTs of different architectures.

To address these challenges, here we describe a series of novel analytic approaches to improve the interpretation of the outcomes of clinical trials deployed to estimate the efficacy of novel TB vaccines. The methods discussed combine *in-silico* tools such as compartmental models and stochastic simulations to disentangle the different possible mechanisms of action underlying vaccine protection effects against TB inferred from trials conducted on either susceptible individuals (IGRA-) or on individuals previously exposed to the pathogen (IGRA+). Our methods unlock he construction of impact forecast metrics that are less subject to bias than previous approaches which typically require the adoption of arbitrary modeling choices about vaccine behavior.

OR38

Diversifying Evolution in *Mycobacterium tuberculosis* and Evasion of Molecular Diagnostics for Isoniazid (INH) Resistance is most prevalent in Asia

S J Valafar [1]
1: Chicago Medical School

Drug resistant Tuberculosis (TB) continues to be a major global concern. INH resistance is commonly first to emerge in treatment of TB. Molecular Diagnostics (mDx) is rapidly becoming an integral component of global TB control. Uncommon mechanisms of resistance escape detection and undermine outbreak containment efforts. In this systematic review, we report all INH-resistance-conferring mutations observed from 2013 through 2019. Overall, 9,306 clinical isolates (5,804 INH^R, 3,502 INH^S) from 31 countries were included. The three most frequently mutated loci continue to be *katG*315 (4,271), *inhA*-15 (787), and *inhA*-8 (106). However, the diagnostic value of *inhA*-8 is far lower than previously thought, only appearing in 25 (0.4%) INH^R isolates lacking the first two mutations. I report 45 new loci (29 *katG*, nine *inhA*, seven *ahpC*) associated with INH resistance and identified 59 loci (common to this and previous reviews) as a reliable basis for mDx. Including all observed mutations provides a sensitivity of 85.6%. In 14.4% of resistant isolates no mechanism of resistance was detected, making them likely to escape mDx, and in case of mono INH-resistance likely to convert to MDR-TB. Integrating the information cataloged in this study into current diagnostic tools is essential for combating the emergence of MDR-TB, and its exclusion can lead to an unintended selection against common mechanisms and to diversifying evolution. Importantly, this diversifying evolution is most frequently observed in Asia, while in Africa, clonal expansion of canonical mutations seems to be most prevalent.

Poster Presenters

P01

Characterization of novel double-reporter strains of *Mycobacterium abscessus* for drug discovery: a study in mScarlet

C M Bento [1 2 3 4] K V Calster [5] T Piller [5] G S Oliveira[1 4] L D Vooght [5] P Cos [5] M S Gomes [1 4] T Silva [1 4]
1: i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal 2: IBMC - Instituto de Biologia Molecular e Celular, Universidade do Porto, Portugal 3: Programa Doutoral em Biologia Molecular e Celular (MCBiology), Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto, Portugal 4: ICBAS – Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto, Portugal 5: Laboratory for Microbiology, Parasitology and Hygiene (LMPH), Wilrijk, Belgium

Treatment of nontuberculous mycobacteria (NTM) disease is based on a long multidrug regimen associated with severe side effects and increased antibiotic resistance. Moreover, in vitro antibiotic susceptibility does not reflect clinical effectiveness, hampering the treatment. Therefore, there is an urgency to find new treatments for NTM infections and better infection models to test them. We have been developing in vitro models for drug susceptibility testing to better recreate the environment the bacteria experience in the host. In this context, we are developing new strains of *Mycobacterium abscessus* which simultaneously express the gene for a fluorescent protein, mScarlet, and the gene for luciferase, which converts D-luciferin in oxyluciferin in the presence of ATP, generating light. The fluorescent signal can be used as a marker of bacterial load and the bioluminescent signal to monitor bacterial metabolism. The strains characterization was made by comparing their growth with the non-transformed strain, measuring, simultaneously, optical density, fluorescence, and luminescence, and correlating those parameters with the bacterial load assessed by colony-forming units assay. We also evaluated the in vitro antibiotic susceptibility of the reporter strains (when compared with the non-transformed), their ability to form biofilms and infect host cells, and validated them for in vivo infection using bioluminescence imaging technology. The results show that these new reporter strains can be an essential tool to aid in the discovery of new drugs against mycobacterial infections.

P02

A quantitative method for the study of HIV-1 and *Mycobacterium tuberculosis* co-infection

S Donnellan [1] S Pennington [2] A Ruggiero [3] C Martinez-Rodriguez [3] M Pouget [3] J Thomas [3] S Ward [2] G Pollakis [3] G Biagini [3] W Paxton [3]
1: Edinburgh Napier University 2: Liverpool School of Tropical Medicine 3: The University of Liverpool

Mycobacterium tuberculosis and human immunodeficiency virus-1 (HIV-1) syndemic interactions are a major global health concern. Despite the clinical significance of coinfection, our understanding of the cellular pathophysiology and the therapeutic pharmacodynamic impact of

coinfection is limited. Here, we use single-round infectious HIV-1 pseudotyped viral particles expressing green fluorescent protein alongside *M. tuberculosis* expressing mCherry to study pathogenesis and treatment. We report that HIV-1 infection inhibited intracellular replication of *M. tuberculosis* and demonstrate the therapeutic activity of antiviral treatment (efavirenz) and antimicrobial treatment (rifampicin). The described method could be applied for detailed mechanistic studies to inform the development of novel treatment strategies.

P03

Drosophila melanogaster as a model for the characterization of host-pathogen interactions of rapid growing *Mycolicibacterium manresensis*.

M Arch [1] M Vidal [1] E Fuentes [1] P J Cardona [1 2]
1: Institut d'Investigació en Ciències de la Salut Germans Trias i Pujol 2: Hospital Universitari Germans Trias i Pujol

The *Drosophila melanogaster* (Dm) has shown to be a good experimental model to study TB, using *Mycobacterium marinum* infection. It has provided an important insight on the innate immunity response, as insects rely solely on this type of response thus avoiding the variability that adaptive mechanisms imply. Also due to the high homology of the genes that determine it with humans: approximately sharing a 75% of genetical homology. We have used this model to characterize how the fast-growing *Mycolicibacterium manresensis*, an environmental, non-pathogenic mycobacteria able to induce a protective immune response against subsequent infections with *Mycobacterium tuberculosis* (Mtb) in mammals, interacts with its host. We have infected systemically both male and females flies with *M. manresensis*, *M. marinum* and *Mycobacterium smegmatis*, a pathogenic and a non-pathogenic mycobacteria respectively. We have then compared the virulence of the three species in this host by assessing the survival, the tissue damage, and the innate immune response of the host. We have also evaluated the replication ability of the three species both in vivo and in vitro using the S2 cell line. Results showed that *M. manresensis* has a high capacity to replicate within the host, kills the flies faster than the virulent species *M. marinum*, and triggers a higher innate immune response in the host.

P04

Phylogenetically related *Mycobacterium tuberculosis* isolates with wild type *rpoB* and rifampicin resistance levels around the critical concentration

P Lempens [1 2] A Mansy [1] S M Arega [1] S Akter [1] M Kaswa [3] C J Meehan [4] B C de Jong [1] L Rigouts [1 2]
1: Institute of Tropical Medicine Antwerp 2: University of Antwerp 3: Programme National de Lutte contre la Tuberculose, République Démocratique du Congo 4: Nottingham Trent University

Rifampicin is the strongest anti-tuberculosis drug, resistance to which is mediated through mutations in the RNA polymerase (*rpoB*) gene. In addition to *rpoB*, other genes potentially associated with *Mycobacterium tuberculosis* rifampicin resistance have been described, although their significance is unclear.

Retrospective whole genome sequencing (WGS) analysis of a set of 324 phenotypically rifampicin-resistant isolates obtained from retreatment tuberculosis patients in Kinshasa, Democratic Republic of Congo, and stored at the Institute of Tropical Medicine Antwerp, showed that 50 (15.4%) isolates had a wild type *rpoB* gene. WGS analysis also revealed that a majority (33/50; 66.0%) of these isolates belonged to *M. tuberculosis* lineage 4.7, all having the same spoligotype (shared international type (SIT) 144). This association between discordant pheno-geno rifampicin results and specific genotypes suggested the evolution of (an) alternative, non-*rpoB* mediated resistance mechanism(s). In this study, further phenotypic and genotypic testing was done to characterise these isolates.

Several mutations in genes previously associated with rifampicin resistance potentially explained resistance in these isolates. Retesting the isolates on Löwenstein-Jensen medium yielded minimum inhibitory concentrations around the critical concentration of 40 mg/L, as did retesting of rifampicin-susceptible isolates of lineage 4.7. The majority of lineage 4.7 isolates tested rifampicin-susceptible in MGIT at the critical concentration of 0.5 mg/L. Future studies using RNA expression profiling could shed light on the relatively modest increase in rifampicin MICs observed in lineage 4.7 not linked to mutations in *rpoB*.

P05

The *Mycobacterium tuberculosis* complex pangenome is small and driven by (sub-)lineage specific regions of difference

M Behruznia [1] M Marin [2] M Farhat [2 3] C J Meehan [1 4]
1: Department of Biosciences, Nottingham Trent University, Nottingham, UK 2: Department of Biomedical Informatics, Harvard Medical School, Boston, MA 02115, USA 3: Pulmonary and Critical Care Medicine, Massachusetts General Hospital, Boston, MA 02114, USA 4: Unit of Mycobacteriology, Institute of Tropical Medicine, Antwerp, Belgium

The pangenome size and function of the *Mycobacterium tuberculosis* complex (MTBC) has been greatly debated in recent years, especially in relation to within-lineage gene content differences. We used a curated dataset including 356 complete genomes from across the MTBC lineages (human and animal) to investigate this gene content variation and identify differential traits between strains that may affect virulence, metabolism, and evolutionary characteristics.

TB-profiler was used for lineage assignment and BUSCO for genome quality control. Genome annotation was performed using PGAP, and Panaroo was used for pangenome analysis. Gene clusters were assigned COG functional categories using eggnog-mapper. Statistical analysis was conducted to examine whether there is a significant association between accessory genome distribution and (sub-)lineage. Multiple whole genome alignment was performed using SibeliaZ to look for sub-lineage regions of differences (i.e., deletions > 10kb in length).

We found a pangenome consisting of 4,115 genes, including 3,636 core genes and a small accessory genome of 479 genes, supporting the clonal evolution of the species. The accessory genome was enriched in transcription, metabolism, and virulence genes whereas the core genome was enriched in genes linked to lipid metabolism and transport, which are essential in host-pathogen interactions. Despite the compact accessory genome size, we identified 296 lineage-specific genes which could explain the differences in metabolic variations and virulence potential. Regions of difference specific to certain sub-lineages were found throughout the MTBC except for lineage 3, indicating that genomic differences exist both between and within the primary lineages.

P06

Effect of Iron and carbon sources on *in-vitro* transcriptional responses to growth arrest of *Mycobacterium tuberculosis*

J A Cárdenas-Pestana [1] S Alebouyeh [2] L Vázquez [2] R Prados-Rosales [2] P Del Portillo [3] M C Menéndez [2] M J García [2] J Sanz [1]
1: University of Zaragoza 2: Universidad Autónoma de Madrid 3: Corporación CorpoGen

The establishment of *Mycobacterium tuberculosis* (Mtb) long-term infection *in vivo* depends on several factors, one of which is the availability of key nutrients such as iron (Fe), and long-chain fatty acids (LCFA). The relation between Fe deprivation inside and outside the granuloma, and the capacity of Mtb to accumulate lipids and persist in the absence of growth is not well understood. The current knowledge of how Mtb modifies its lipid composition in response to growth arrest, depending on iron and lipids availability is scarce. To explore these topics, we compare genome-wide transcriptomic profiles of Mtb at exponential and stationary growth phases using cultures with glycerol–dextrose, glycerol, and LCFA as the carbon sources, in the presence or absence of iron. We focused on comparing Fe effects on response to growth arrest with either glycerol–dextrose or glycerol constituting the main carbon source for the bacteria; and the effect of shifting from glycerol and dextrose as main carbon sources to LCFA in the presence of Fe. We found that transcriptomic responses to growth arrest, considered as the transition from exponential to stationary phase are enhanced when culture conditions incorporate nutrient cues characteristic of the phagosomal environment such as low levels of iron and high concentration of LCFAs. Effects of low iron levels and LCFA on responses to growth arrest are significantly correlated and impact key pathways to bacterial survival upon phagocytosis such as energy production and stress responses, suggesting a convergent signaling dynamics of these cues towards the dormant phenotype.

P07

Multi-omics portrait of VirR protein function in cell wall remodeling and vesiculogenesis in Mycobacterium tuberculosis

J Bertol [1] V Salgueiro [2] A Palacios [3] L Lerma [2] S Alebougeh [2] L Sampedro [3] J L Lavin [4] F Elortza [3] M Azkalgorta [3] J Anguita [3] I Marchante [1] J Sanz [1] R Prados-Rosales [2]
1: University of Zaragoza 2: Autonomous University of Madrid 3: CIC bioGUNE 4: Neiker - Basque Institute for agricultural research and development

VirR, a LytR_C domain containing protein, has been related to regulation of cell wall integrity and production of extracellular vesicles (EVs) in *Mycobacterium tuberculosis*. To test its regulatory role in vesiculogenesis, we obtained proteomic and transcriptomic profiles from a virR-KO mutant strain of *M. tuberculosis*, as well as from the wild-type strain H37Rv. The VirR-KO mutant presented an abnormal cell wall morphology linked to higher permeability, lower virulence and an increased production of EVs.
Transcriptomic analysis was performed on whole cell lysates from each strain revealing major

differences concerning host-induced stress responses, as well as protein secretion, which partly explain the divergent secretory and virulent profiles of the wild-type and mutant strains. Furthermore, these analyses also revealed divergent expression levels for genes involved in post-transcriptional regulation, hinting at a role for VirR in these processes. To test this hypothesis, proteomic analyses were performed on whole cell lysates and isolated extracellular vesicles from each strain, revealing largely disjoint sets of differentially expressed proteins with respect to mRNAs, further pointing to a post-transcriptional regulatory role for VirR. Differential expression analysis of the proteins between the WT and the VirR mutant revealed greater differences in EVs than in whole cell lysates, indicating a primary role for VirR in the proteomic enrichment profile of EVs.
Taken together, our results highlight a relevant regulatory role for virR that leans both on transcriptional and post-transcriptional mechanisms, which significantly mediates the amount and function of EVs secreted by *Mycobacterium tuberculosis*.

P08

Mutation rates in strains of different *Mycobacterium tuberculosis* lineages associated with emergence of multi-drug resistant tuberculosis

E Rousseau [1] T Wirth [2 3] M Merker [4] S Niemann [1 5]
1: Molecular and Experimental Mycobacteriology, Research Center Borstel, Borstel, 23845, Germany 2: EPHE, PSL University, Paris, 75014, France 3: Institut de Systématique, Evolution, Biodiversité, ISYEB, Muséum national d'Histoire naturelle, CNRS, Sorbonne Université, EPHE, Université des Antilles, Paris, 75005, France 4: Evolution of the Resistome, Research Center Borstel, Borstel, 23845, Germany 5: German Center for Infection Research, Partner site Hamburg-Lübeck-Borstel-Riems, Borstel, 23845, Germany

Antibiotic resistances are unequally distributed among strains of different *Mycobacterium tuberculosis* complex (MTBC) lineages. Strains belonging to the two most worldwide prevalent generalist lineages 4 (L4) and 2 (L2) have caused large outbreaks of multidrug resistant tuberculosis (MDR-TB) and in certain cases, those MDR-TB clades evolved toward the acquisition of resistances against additional MDR-TB drug. However, certain mechanisms and the resistance acquisition pace contributing to the success of those strains remains unclear.

Here, we experimentally determined resistance acquisition rates for rifampicin and bedaquiline in a collection of clinical L2 and L4 strains MTBC strains using fluctuation assays and whole-genome sequencing.

Modern L2 strains acquire spontaneous rifampicin resistance mutations twice faster than L4 strains *in vitro*. Most frequently acquired rifampicin resistance mutations were C1333T (His445Tyr), A1334G (His445Arg) and C1322T (Ser441Leu). All strains investigated acquired known resistance conferring mutations; no particular lineage specific mutation hotspot was identified. Preliminary results indicate that L2 strains also acquire bedaquiline resistances faster than L4 strains.

Our results indicate that the genetic background of modern L2 strains potentially allows for an accelerated spontaneous mutation rate leading to a faster resistance acquisition of modern L2 strains. The genetic make-up may also reduce fitness costs of drug resistance and/or favors the acquisition of compensatory mutations.

P09

Rifampicin tolerance and growth fitness among isoniazid-resistant clinical *Mycobacterium tuberculosis* isolates: an in-vitro longitudinal study.

S Vijay [1 2 3] N L H Bao [1] D N Vinh [1] L T H Nhat [1] D D A Thu [1] N L Quang [1] L P T Trieu [1] H N Nhung [1] V T N Ha [1] P V K Thai [6] D T M Ha [6] N H Lan [6] M Caws [4] G E Thwaites [1 2] B Javid [5] N T T Thuong [1 2]
1: Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam 2: Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom 3: Theoretical Microbial Ecology, Friedrich Schiller University, Jena, Germany 4: Department of Clinical Sciences, Liverpool School of Tropical Medicine, Liverpool, United Kingdom 5: Division of Experimental Medicine, University of California, San Francisco, California, USA 6: Pham Ngoc Thach Hospital, Ho Chi Minh City, Vietnam

Antibiotic tolerance in *Mycobacterium tuberculosis* leads to less effective bacterial killing, poor treatment responses and resistant emergence. Therefore, we investigated the rifampicin tolerance of *M. tuberculosis*, with or without isoniazid-resistance. Rifampicin survival fraction was determined by minimum duration of killing assay in isoniazid susceptible (n=119) and resistant (n=84) isolates. The longitudinal isoniazid-resistant isolates were analyzed for rifampicin tolerance based on collection time from patients and associated emergence of genetic variants. The median duration of rifampicin exposure reducing the *M. tuberculosis* survival by 90% (minimum duration of killing-MDK90) increased from 1·23 (95%CI 1·11; 1·37) and 1·31 (95%CI 1·14; 1·48) to 2·55 (95%CI 2·04; 2·97) and 1·98 (95%CI 1·69; 2·56) days, for isoniazid-susceptible and resistant respectively, during 15 to 60 days of incubation respectively. Increase in MDK90 time indicated the presence of fast and slow growing tolerant sub-populations. A range of 6 $\log_{10}$-fold survival fraction enabled classification of tolerance as low, medium or high and revealed isoniazid-resistance association with increased tolerance with faster growth (OR=2·68 for low vs. medium, OR=4·42 for low vs. high, *P*-trend=0·0003). The high tolerance in longitudinal isoniazid-resistant isolates was specific to those collected during rifampicin treatment in patients and associated with bacterial genetic microvariants. Our study reveals that isoniazid resistance is associated with higher tolerance with growth fitness. Furthermore, rifampicin treatment may select isoniazid-resistant isolate microvariants with higher rifampicin tolerance, with survival potential similar to multi-drug resistant isolates. These findings suggest that isoniazid-resistant tuberculosis needs to be evaluated for rifampicin tolerance or needs further improvement in treatment regimen.

P10

Non-redundant Pangenome construction of Mycobacterium tuberculosis

Y ZHOU [1 2] R Anthony [3] D Soolingen [2 3] Y zhao [1]
1: chinese center for disease control and prevention 2: Radboud University MC 3: RIVM

A pangenome provides a more complete overview of the genetic content of a clade than an individual strain's genome. However, pangenome analysis is time-consuming and resource-intensive, and a standardized pipeline is lacking compromising reproducibility and reliability. We investigated the pangenome of 421 epidemic Mycobacterium tuberculosis strains in China. We

produced assemblies using SPAdes with data from the Illumina platform excluding short contigs (<200 bp) and contaminations. CDSs were annotated using Prokka and homologues clustered using get_homologues. Redundant genes were merged for clusters with > 90% identity and > 90% alignment and genes overlapping >60 bp filtered using in-house scripts. The COG and OMCL algorithms identified 7702 and 7995 homologues clusters, respectively. After merging redundant genes, there were 5071 clusters left, and >98% of the merged clusters had less than 450 entries in each cluster. Among these 5071 clusters, 1165 clusters were not clustered with Rv genes. We checked the overlap of these clusters with Rv genes and excluded 575 clusters at this step. Ignoring any mutations in genes, the final pangenome for the 421 genomes consists of 4574 genes/ORFs, including 2980 core genes, 1117 softcore genes, 198 shell genes, and 279 cloud genes. The 590 new genes include 106 core genes, 80 soft-core genes, 132 shell genes, and 272 cloud genes. 184 of these “new genes” are present in the H37Rv genome but are not considered as CDSs in the latest available annotation data. Heap’s index is 1.000013, indicating this sample set of 421 genomes has a closed pangenome.

P11

Comparison of 13 software tools to detect structural variation in *Mycobacterium tuberculosis*

Y Zhou [1 2] D Soolingen [2 3] R Anthony [3] Y zhao [1]
1: China CDC 2: Radboud University MC 3: RIVM

Studying structure variations using whole genome sequencing data is challenging due to the lack of reliable tools. To detect deletions, we applied 13 software to 420 Mycobacterium tuberculosis strains sequenced on the Illumina platform. Intersection between different methods was used to measure quality. By investigating the composition of deletion length, the methods could be divided into 4 groups. Group 1 has 5 methods (breakdancer, cnvnator, delly, lumpy-smoove, tiddit), which detected deletions mostly (61~95%) with a length between 100-1000 bp. Group 2 has 4 methods (assemblytics , unimap, pindel, svaba), which detected deletions mostly (68~88%) with a length less than 100 bp. Group 3 has 2 methods (manta , softsv), which detected a large proportion (88% and 30%, respectively) of large deletions (> 100k bp). Group 4 only has utilizes bcftools, which only detected deletions less than 100 bp. Deletions longer than 100k were excluded for subsequent analysis. For each sample, group 1 methods detected 10 to 50 deletions in most samples, group 2 between 110 to 180, group 3 between 50 to 100. Bcftools shows two peaks, one is at about 10 deletions, and the other is 60 deletions. The later peak contains mostly (104/107) Beijing family strains. Group 2 methods show the highest concordance as suggested by intersection proportion (77~95%). Our study suggests that detection of deletions less than 100 bp is mostly reliable using bcftools, pindel, unimap, or svaba. For deletions longer than 10k bp, these 13 methods are not reliable (with Illumina data).

P13

Assessment of circulating mitochondrial cell-free DNA dynamics in patients with tuberculosis: pilot study

L Freimane [1] A Viksna [2] V Ulanova [1] A Kivrane [1] D Sadovska [1] R Ranka [1]
1: Latvian Biomedical Research and Study Centre, Riga, LV-1067, Latvia 2: Centre of Tuberculosis and Lung Diseases, Riga East University Hospital, Riga, LV – 2118, Latvia

Circulating cell-free (ccf) mtDNA copy number (CN) variations can indicate physical injury, inflammation, innate immunity system activity, and predict mortality and treatment outcome in critically ill individuals. Also, pathogen-derived ccf DNA, including *M. tuberculosis* (*Mtb*), has been detected in humans, which could directly show infection load and serve as biomarker for disease monitoring.

In this study, blood samples were collected from ten newly diagnosed patients with *Mtb* infection before treatment and after every two weeks for two months. For ccf-mtDNA and ccf-*Mtb* CN evaluation DNA was extracted respectively from (a) 200 µL freshly frozen blood plasma, and (b) 500 µL whole blood samples. Absolute CN (CN/µL of plasma/blood) was quantified using the QX200 droplet digital ddPCR System (Bio-Rad) via two separate multiplex amplifications within *ND1* and *CYB* (mtDNA), and *IS16110* and *CFP-10* (*Mtb*) gene regions. QuantaSoft software (1.0.596) was used to analyse the data. Statistical analysis was performed using GraphPad Prism 5.0 software.

Almost all patients showed extremely high ccf-mtDNA plasma concentrations (maximum of 28163 CN/µL before therapy); we could detect ccf-mtDNA CN fluctuations in time which were statistically significant in those patients which showed CN decrease in the first two weeks of the therapy (p=0.03). We detected ccf-*Mtb* only in one patient before treatment with concentration of 0.023 CN/µL.

In conclusion, ccf-*Mtb* CN wasn't informative in our settings due to extremely low concentrations. Ccf-mtDNA may serve as a useful biomarker for *Mtb* infection monitoring, but further studies are necessary. This study was supported by ERDF grant No. 1.1.1.1/20/A/046.

P14

Evaluating immunological parameters in human fluids for diagnostic purposes in Tuberculosis.

G Gloria Guerrero M [1] A Luis Alejandro Aguilera Galaviz [1] M Aurora Martinez Romero [2] F Juan Manuel Favela-Hernandez [2]
1: University Autonome of Zacatecas 2: University Juarez of Durango State 3: Multidisciplinary Institute of Sciences AVICENA de Torreon Coahuila

[4]Instituto Avicena. Torreón, Coahuila. México

Mycobacterium tuberculosis (MTb), which causes human tuberculosis, an intracellular Gram-positive pathogen, remains a "hot spot" in public human health since approximately 1.7 million deaths annually and morbidity of 10.6 million. Most of the infected individuals remain asymptomatic during most of their life, while only 5 % develop active disease. A concern is the

continuous uprising in multi-drug drug-resistant strains (MDR). An issue that is the subject of intense research is how the development and improvements of molecular and immunological techniques can aid in diagnosis and better therapeutic treatments. In recent years, the combined use of molecular, microbiological, and imaging methods has given promising results. In previous work, we determined a set of biomarkers using microarray technologies. Serological reactivity to *M. bovis* or *M. tuberculosis* was also evaluated. Therefore, in the present work, we focused on assessing immunological parameters (cytokines, and antibodies) to measure the interaction host-pathogen and for diagnostic purposes.

P15

GENOTUBE, a high-throughput tool for exploring the genetic diversity of pathogenic mycobacteria

A Le Meur [1] F Hak [1] R Z Eddine [2 3] C Sola [4 5] G Refregier [1]
1: Université Paris-Saclay, CNRS, AgroParisTech, Ecologie Systématique et Evolution, 91190, Gif-sur-Yvette, France 2: Al Afak institute for health Sciences, Liban 3: Laboratoire d'Optique et Biosciences (LOB), Ecole Polytechnique, Route de Saclay 91120, Palaiseau, France 4: INSERM-Université Paris-Cité, IAME Laboratory, UMR1137 5: Université Paris-Saclay, 91190 Saint-Aubin, France

More than 100,000 *Mycobacterium tuberculosis* sequence reads (SRA) are publicly available. To make use of this resource, adapted analysis tools are required to mitigate analysis timeframe and storage space.

We developed a NGS data analysis pipeline to explore *Mycobacterium tuberculosis* strains' polymorphisms, with an option to restrict the analysis to genes of interest. This pipeline is called genotube for GENe Oriented TUBErculosis pipeline. It has been developed with Nextflow process management software and is containerized to ease installation and provide reproducible results. Genotube is available on github. We benchmarked our tool with artificial genomes include all steps of evolution known to apply to this pathogen such as Single Nucleotide Variations, indels, IS*6110* transposition, large deletions, as well as duplications.

GENOTUBE integrates the classic steps of a genomics pipeline (downloading, mapping, variant calling), as well as classic steps in *M. tuberculosis* all-in-one pipelines such as lineage and sub-lineage identification, *in silico* prediction of antibiotic resistance). In addition, GENOTUBE includes several phylogeny modules (tree construction, ancestral character inference, reversion and homoplasy identification). GENOTUBE proved more rapid, sensitive and specific than other tools such as Phyresse, MTBseq and TBProfiler. In addition, we identified that the risk of wrong inferences regarding SNVs is inflated at the border of deletions, IS*6110* insertions or deletions, and duplications.

To sum up, GENOTUBE is a new tool to extract the diversity at loci of interest from large SRA samples of *M. tuberculosis*. It eases the detection of genetic associations. It can be adapted to other bacteria.

P16

Demonstration of nanopore sequencing for the detection of tuberculosis and other infectious diseases in low-and-middle-income countries

A A Slyzkyi [1] E Tiemersma [1] B Myrzaliev [1] L Mtei [1] H Nguyen [1] A van den Bout [4] L Manrho [4] N Hermans [4] K Stoffers [4] E Ubbelohde [1 3] P Lempens [1] C Mulder [1 2] R Anthony [3] J de Beer [4] A van der Zanden [4] K Kremer [1]
1: KNCV Tuberculosis Foundation 2: Amsterdam Institute for Global Health and Development (AIGHD) 3: National Institute for Public Health and the Environment 4: Laboratory for Microbiology Twente Achterhoek (Labmicta)

Genome sequencing has been used mainly in large institutes for research and surveillance purposes, but recent innovations, such as the portable and low-cost MinION devices (Oxford Nanopore Technologies), enable genome sequencing close to point-of-care. We aim to demonstrate the use of nanopore sequencing with MinION devices for the diagnosis of tuberculosis (TB), COVID-19, other infectious diseases, and AMR in Kyrgyzstan, Tanzania, and Vietnam.

Training of project country staff has taken place, study protocols were developed, and procurement and importation procedures are ongoing. Nanopore sequencing studies at national level laboratories in the project countries will start in Q2, 2023. Subsequent implementation at decentralized settings follows later.

In the Netherlands, assay development and validation experiments were done. An optimized approach for nanopore sequencing of SARS-CoV-2 was developed. It achieved sufficient and more equally distributed coverage of the SARS-CoV-2 genome. An in-house TB assay was designed and compared by using MinION sequencing with two commercial assays developed for Illumina sequencing. The assays performed well on strains, but showed variable coverage of certain resistance genes in sputum samples. In addition, nanopore sequencing of mixtures of DNA of a multidrug resistant TB strain and a susceptible strain by using the Deeplex Myc-TB (Genoscreen) assay showed that mixed infections could be detected down to 10% abundance.

We aim to generate evidence on the effectiveness, feasibility, acceptability, and costs of using nanopore sequencing for the diagnosis of TB, other infectious diseases, and AMR. Challenges and critical issues will be highlighted guiding future development and scale up.

P17

Early positive culture: what is in a name?

E C Conceicao [1] F Wells [1] M Ismail [1] B Mann [1] J T N Ngom [1] S Omar [1] A Dippenaar [1 2] A Van Rie [2] R Warren [1]
1: South African Medical Research Council Centre for Tuberculosis Research, Division of Molecular Biology and Human Genetics, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa 2: Department of Family Medicine and Population Health, Global Health Institute, Faculty of Medicine and Health Sciences, University of Antwerp, Antwerp, Belgium

Obtaining quick culture results for *Mycobacterium tuberculosis* is crucial for effective patient care when utilizing culture-dependent diagnostic assays. The BD BACTEC™ MGIT™ 960 system is a culture method that flags positive at >100 Growth Index (GI), corresponding to 10^5 to 10^6 colony-forming units per millilitre. In 1988, Ellner introduced the concept of “early detection” when applying molecular identification methods to mycobacteria growth indicator tubes (MGITs) with GI<100. Recently, culture-based whole-genome sequencing (WGS) for clinical care renewed the interest in primary MGIT cultures to eliminate the sub-culture step. In 2015, Votintseva published a standardized protocol for DNA extraction for WGS from clinical isolates and introduced the term “early positive liquid (MGIT) cultures” (EPC). Since then, many investigators have used EPCs for next-generation sequencing applications. We performed a systematic literature review to map the definitions used for EPC. Of 423 potential studies, 15 were eligible. None of the 15 studies performed DNA extraction immediately after MGITs flagged positive. Most batched isolates, resulting in a combination of true “EPCs” and MGITs with continued growth. Only one study defined the term EPC as “1 to 7 days after positivity and re-incubation”. All other studies failed to report the length of the incubation period between flagging positive and DNA extraction. Because the crucial distinction is between the use of a primary culture versus a sub-culture, we propose to replace EPC with ‘clinical primary MGIT culture’. Future studies should determine the optimal balance between continued growth in MGIT for increased DNA yield and turn-around time.

P18

Evaluation of whole-genome sequencing of *Mycobacterium tuberculosis* isolates in clinical laboratory

E Sodja [1] S Truden [1] M Žolnir-Dovc [1]
1: University Clinic Golnik

Rapid development of whole-genome sequencing (WGS) has facilitated the interpretation of genetic patterns underlying phenotypic resistance in *Mycobacterium tuberculosis* (MT). To evaluate WGS in Laboratory for Mycobacteria (University Clinic Golnik, Slovenia), we retrospectively selected 66 MT isolates with suspected drug resistance from the National Mycobacterial Strain Collection in years 1996-2020. Additionally, 125 MT isolates from years 2021-2023 were prospectively included.

In retrospective cohort with high percent of resistant MT isolates (64/66; 97%), we observed different levels of agreement between phenotypic drug susceptibility testing (pDST) and WGS for different antibiotics (rifampicin 98.5%, ethambutol 92.3%, pyrazinamide 90.9%, ethionamide 84.1%, isoniazid 81.8%). One possible explanation for lower agreement between methods for detecting isoniazid resistance is that 9/66 (14%) were phenotypically sensitive to INH, where promoter mutation–57c>t in *oxyR-ahpC* was detected using WGS. For other antibiotics, such comparison was limited due to low number of MT isolates tested with pDST and due to detection of lesser-known mutations with yet unknown impact on resistance.

Prospectively, only six MT isolates (6/125; 5%) harboured different mutations linked to drug resistance. Resistance for all six MT isolates was confirmed with pDST leading to 100% with WGS. Most prevalent mutation was c-15t in *fabG1*, which was detected in four MT isolates with ETH and low level INH resistance.

To summarize, WGS proved to be useful in detecting resistance at the least for the first line antibiotics. To strengthen the power of WGS, the inclusion of isolates from different geographical regions in WHO mutation catalogue is necessary.

P19

Implementation and evaluation of the ABL-DeepChek 13-Plex Assay for diagnosis of antibiotic resistant tuberculosis

V Mohr [1 2] C Utpatel [1 2] C Sayada [3] S Niemann [1 2]
1: Molecular and Experimental Mycobacteriology, Research Center Borstel, Germany 2: National Tuberculosis Reference Laboratory, Research Center Borstel, Germany 3: ABL Diagnostics S.A, France

Introduction:

Emerging resistances against anti Tuberculosis (TB) drugs are a major risk to global health security. Therefore, rapid resistance detection is crucial for effective and successful treatment and thereby essential to prevent transmission. A promising alternative, solving the major problems of phenotypic drug susceptibility testing (pDST) and Whole Genome Sequencing (WGS), is targeted next generation sequencing (tNGS) allowing for fast, sensitive and high-throughput decoding of MTBC strains genetic information. Here we present the evaluation of the ABL-DeepChek 13-Plex Assay for diagnosis of antibiotic resistant Tuberculosis (ABL Diagnostics S.A.), on the basis of detected mutation profiles.

Methods:

The concordance of detected mutations as well as resistance predictions is determined by comparing tNGS data to WGS data. For the data analysis both the ABL BacterioChek web tool and a modified MTBseq pipeline are used to independently verify and compare the results.

Results:

Of all in BacterioChek detected mutations, 567 (94.4%) were concordant to the WGS data. Furthermore, resistance inference of the ABL-DeepChek 13-Plex Assay 1.2, starting from culture gDNA, matched the predictions from WGS in 1146 (89.1%) observations. For DNAs from sputum

specimens, 450 (90.7%) predictions agreed. Discrepancies were mainly based on different resistance prediction catalogues and rules.

Discussion:

tNGS with the DeepChek Assay 13-Plex KB Drug Susceptibility Testing v.1.2 has the potential to replace pDST for a large fraction of patients. It is an end-to-end solution for rapid resistance and drug susceptibility testing in TB diagnostics.

P21

Exploring the potential of Oxford Nanopore Technologies for *Mycobacterium tuberculosis* sequencing: an assessment of R10 flowcells and V14 chemistry

A Dippenaar [1] E Costa Conceição [2] F Wells [2] J Loubser [2] B Mann [2] M De Diego Fuertes [1] V Rennie [1] R M Warren [2] A Van Rie [1]
1: University of Antwerp 2: Stellenbosch University

Oxford Nanopore Technologies (ONT) could decentralize *Mycobacterium tuberculosis* (*Mtb*) sequencing but the need for high (≥50 ng) DNA concentrations and suboptimal accuracy prevented its implementation. We assessed the performance of ONT R10 flowcells and V14 rapid barcoding chemistry for *Mtb* whole genome sequencing of DNA extracted from clinical primary liquid cultures (CPLCs). Using the recommended protocol for MinION Mk1C, R10.4.1 MinION flowcells and ONT Rapid Barcoding Kit V14 on six CPLC samples, the pooled library yield was 10.8 ng/ul, 25Gb of data and 214k reads were generated after 48h, but 49% of reads failed to meet the Phred Quality score threshold (>8). Because we hypothesized that the poor performance was due to impurities in CPLCs, we added a pre-library preparation bead-cleanup step. The yield for four CPLCs and one *Mtb* subculture (control) was similar (10.8 ng/ul), and 32Gb of data and 822k reads were produced but the quality remained poor (66% reads with Phred Quality >8). A Phred Quality score >20 (modal accuracy above 99%) was only achieved for samples that underwent bead-cleanup. A third run of five CPLCs with bead clean-up only produced 12Gb of data, 166k reads of which only 51% achieved Phred Quality score >8. Using TB-profiler, a median depth of coverage above 10x was only achieved in four of 17 sequenced libraries and drug resistance profiles could not be determined. These results suggest that further optimization of the latest ONT rapid barcoding chemistry and library preparation protocol is needed before nanopore sequencing can guide tuberculosis care.

P22

Beyond the clone: A new ancestor for *Mycobacterium tuberculosis* and implications for host-specific genomic events

P Ruiz-Rodriguez [1] M Coscolla [1]
1: I2SysBio, University of Valencia-FISABIO Joint Unit, 46980 Paterna, Spain

Mycobacterium tuberculosis is an exceptionally virulent pathogen, often characterized as a monomorphic bacterium. In this study, we aimed to investigate the genetic diversity within the *M. tuberculosis* Complex (MTBC) to gain deeper insights into its pathogenicity. Although the MTBC has limited diversity, it comprises lineages that infect humans (L1-9) as well as those predominantly infecting animals (A1-4). Traditional genetic studies have primarily relied on Illumina mapping and a reference *M. tuberculosis* ancestor based on the H37Rv strain (L4), which might result in an incomplete representation of the full MTBC spectrum.

To gain a better understanding of MTBC genomic diversity, we constructed a comprehensive MTBC pangenome using 340 complete whole genome sequences and via graph algorithm. We applied maximum-likelihood and Bayesian inference techniques to reconstruct the ancestral sequence of MTBC.

We reconstructed a new, refined annotation for the MTBC ancestor that we defined as pancestrome (the pangenome of the ancestor). We corrected inaccuracies in the annotation caused by software annotators, using the curated annotation of H37Rv. We discerned patterns of gene presence and absence across MTBC lineages, capturing ancestral diversity leading to each clade. Additionally, we described within- and between-lineage diversity through SNP accumulation, and indels. Moreover, we identified evolutionary selective pressures differentiating lineages within the complex using all genes and epitopes identified in the pancestrome. Our results offer a new reference and annotation capturing whole MTBC for genomic analysis and highlight the potential of host-specific genomic markers to identify unique virulence factors associated with specific hosts.

P23

Determining the situation of drug-resistance Tuberculosis in the South of Mozambique by using whole genome sequencing for the first time

C Mariner-Llicer [1] B Saavedra-Cervera [2 3] E Mambuque [3] S Munguambe [3] N Gomes [3] I Cancino-Muñoz [5] L Villamayor [4] M Torres-Puente [1] D Nguenha [3 6] D Respeito [3] G Tembe [3] M G López [1] I Comas [1 7] A García-Basteiro [2 3 8]
1: Instituto de Biomedicina de Valencia (CSIC) 2: ISGlobal, Hospital Clínic - Universitat de Barcelona 3: Centro de Investigação em Saúde de Manhiça (CISM), Maputo, Mozambique 4: FISABIO Public Health, Valencia, Spain 5: Universitat de València, Valencia, Spain 6: Amsterdam Institute for Global Health & Development (AIGHD). Amsterdam, The Netherlands 7: CIBER in Epidemiology and Public Health, Madrid, Spain 8: Centro de Investigación Biomédica en Red de Enfermedades Infecciosas (CIBERINFEC), Barcelona, Spain

Mozambique is considered a high multidrug-resistant (MDR-TB) country by the World Health Organization (WHO) (3.7% new MDR-TB cases). The last national WHO survey was performed in 2008. Nowadays, South Africa and Eswatini are reporting an increase in MDR-TB cases heightened, in Eswatini, by the spread of a MDR-TB strain harboring the rpoB_I491F mutation not detected by XpertUltra/RIF. Due to the concerning MDR-TB situation in Southern Africa, it is crucial to review the current resistance status in Mozambique. Our aim is to depict the current evolution of drug-resistance prevalence to first and second-line drugs compared to 2008. We analyzed 275 and 337 genomes from two population-based surveys conducted in Manhiça in 2014 and 2018 and looked for drug-resistance mutations included in the recently published WHO catalog. We found that the overall resistance increased slightly from 10.78% to 14.42%. Although, new multidrug/rifampicin resistance cases remained consistent with the 2008 prevalence study (3.5%) indicating that MDR-TB is not spreading as rapidly as in neighboring countries. Importantly, we detected a high isoniazid (INH) prevalence not associated with MDR-TB (4.20% and 7.61% in 2008 and 2018, p-value=0.03) suggesting that a sizeable number of cases are INH-resistant before starting treatment and not detected by XpertUltra/RIF. Fortunately, no mutations associated with second-line drug-resistance were found in the dataset. Our results show a stable drug-resistance situation in Manhiça with the need to monitor isoniazid resistance, and highlight the potential of WGS to be used in national surveys to expand our knowledge of drug-resistance prevalence throughout all Mozambican provinces.

P24

MAGMA – A novel bioinformatics pipeline developed for integration of WGS in clinical care and tuberculosis control

T H Heupink [1] L Verboven [1] A Sharma [2] V Rennie [1] R M Warren [2] A Van Rie [1]
1: Family Medicine and Population Health (FAMPOP), Faculty of Medicine and Health Sciences, University of Antwerp, Wilrijk, Belgium 2: DSI-NRF Centre of Excellence for Biomedical Tuberculosis Research, South African Medical Research Council Centre for Tuberculosis Research, Division of Molecular Biology and Human Genetics, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa

Mtb bioinformatics pipelines developed for research purposes often struggle to analyze *Mycobacterium tuberculosis (Mtb)* DNA from clinical samples with low mycobacterial burden and high levels of contamination. Building on the XBS variant calling core, we created the open-source MAGMA (Maximum Accessible Genome for Mtb Analysis) pipeline. MAGMA is implemented in Nextflow, employs the strengths of various software packages, and is compatible with various computing systems. After quality control (median coverage ≥10x, coverage breadth <90%, mixed infections or NTM frequency <20%), the pipeline starts with an individual sample workflow where minor, major and structural variants are identified. Major variants are subsequently called at cohort level and filtered using a machine learning approach. The accurate identification of variants in both regular and complex regions of the Mtb genome increases the genetic resolution by 9%. TBProfiler is implemented for lineage identification and drug resistance profiling of the major and minor variants. ClusterPicker is used for cluster identification based on 5 and 12bp SNP cut-offs. IQtree is employed to construct a Maximum Likelihood phylogeny and iTOL annotations for visualizing of the phylogenetic tree. A case study showed that MAGMA accurately analyzes data from early positive primary cultures with variable *Mtb* DNA to total DNA (mapping percentage 8% to 87% and median coverage 57x) and large amounts of contaminating sequences (poly-modal GC content distribution). MAGMA accurately analyses clinical *Mtb* samples and provides users with a range of data and visual outputs that can guide precision medicine and precision public health interventions.

P25

Rapid detection of IS*1081* of *Mycobacterium bovis* using CRISPR/Cas12a system combined with recombinase polymerase amplification

S H Son [1] J H Lee [1] H Y Lee [1] S S Yoon [1] J S Choi [1]
1: Animal and Plant Quarantine Agency

Mycobacterium tuberculosis variant *bovis* (*M. bovis*), a member of *Mycobacterium tuberculosis* complex, is the most important causative agent of tuberculosis (TB) in cattle. It has a wide host range and can also cause TB in human through zoonotic transmission. Although it is considered less serious in some developed countries, zoonotic TB caused by *M. bovis* infection is a considerable public health threat in areas where milk is frequently consumed without heat-

treatment or close human-animal contact is common. In order to prevent the spread of zoonotic TB, it is necessary to quickly identify cattle infected with *M. bovis*. Despite the rapid diagnostic platform using sputum for human TB have already been distributed, it has not been evaluated for animal-derived samples and the culture and identification of *M. bovis* are still the gold standard test for bovine TB diagnosis. For rapid detection of *M. bovis*, we developed a clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) 12a system-based assay combined with recombinase polymerase amplification (RPA) detecting insertion sequence element IS*1081*, one of the widely used genetic markers for *M. bovis* detection. Using this detection method, the presence of IS*1081* in genomic DNA of *M. bovis* AN5 and BCG strains could be confirmed within 40 min and none of the false-positive reactions for 19 other bacteria including 11 nontuberculous mycobacteria (NTM) strains were shown. Although further evaluation on clinical samples is required, the RPA-CRISPR/Cas12a assay may contribute to zoonotic TB control through more rapid, simple and accurate detection of *M. bovis*.

P26

Spatial clustering of rifampicin-resistant tuberculosis and dominant clone in Rwanda: implications for targeted case finding

I Cuella Martin [2] J C.S Ngabonziza [2 3 4] W Mulders [2] L Rigouts [2 5] B C de Jong [2] E M.H Mitchell [1]

1: Department of Public Health, Institute for Tropical Medicine, Antwerp, Belgium 2: Mycobacteriology Unit, Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium 3: Research Innovation and Data Science Division, Rwanda Biomedical Centre, Kigali, Rwanda 4: Department of Clinical Biology, University of Rwanda, Kigali, Rwanda 5: Department of Biomedical Sciences, Antwerp University, Antwerp, Belgium

In 2005, Rwanda established a program to manage rifampicin-resistant tuberculosis (RR-TB), which included standardized treatment and drug-susceptibility testing for rifampicin. Since then, the estimated prevalence of RR-TB in new cases declined from 3.9% (95%CI 2.5-5.7%) to 1.4% (95%CI 1.09-1.89%) in 2019-2020. RR-TB in Rwanda is largely driven by transmission of a single dominant clone, the Rwanda RR-TB clone (R3clone), which is resistant to all first-line drugs. In order to pinpoint recent transmission chains and develop effective targeted case-finding strategies, we utilized spatial epidemiology to detect any remaining hotspots.

We conducted a retrospective analysis of 213 patients from 2005-2021 with RR-TB confirmed through whole genome sequencing (WGS) and a known geographical cell address (Rwanda is comprised of 2148 cells). 52% (112 of 213) of the included isolates were R3 clone. Total RR-TB and R3clone prevalence were mapped using QGIS, and SaTScan software was used with the discrete Poisson model to identify circular clusters.

The study of RR-TB cases revealed the presence of three significant spatial clusters comprising 91 cells in the vicinity of Kigali, and exhibiting relative risks of 5.76, 10.30 and 11.59. The analysis of R3 clone hotspots showed again three Kigali clusters, as well as an additional cluster of eight cells situated in Huye district (Southern Province). These findings suggest that R3 clone may have a higher degree of transmissibility. Future analyses will explore possible diagnostic access, demographic, and health correlates. This study highlights the importance of continued monitoring and analysis to target more effective prevention and control measures.

P27

Evaluating the performance of the novel Xpert MTB/XDR assay in detecting fluoroquinolone hetero-resistance

A Dippenaar [1 2] M Diels [1] J Keysers [1] W Mulders [1] E Ardizzoni [1] O Tzfadia [1] S Cogneau [1] P Rupasinghe [1] A Van Rie [2] B de Jong [1] L Rigouts [1 2]
1: Institute of Tropical Medicine Antwerp 2: University of Antwerp

Timely detection of fluoroquinolone resistance is essential for the effective treatment of rifampicin-resistant tuberculosis. Hetero-resistance, the co-occurrence of drug-susceptible and drug-resistant populations, can be difficult to detect by molecular methods when the drug-resistant bacilli are a minority population. We aimed to determine the accuracy of the novel GeneXpert MTB/XDR assay to detect fluoroquinolone hetero-resistance caused by the *gyrA* D94G variant, a common variant conferring high-level fluoroquinolone resistance. We used a *gyrA* and *gyrB* wild-type strain and its isogenic *gyrA* D94G daughter strain to generate quality-controlled hetero-resistant mixtures. The isolates were plated for colony counting and mixed at different ratios (0.5%-100% resistant). The ratio of resistant to susceptible populations was then determined genotypically by Deeplex-MycTB. The mixtures were assessed phenotypically by minimum inhibitory concentration testing using EUCAST broth microdilution. The mixtures are available as thermolysates in the Belgian Coordinated Collections of Microorganisms (www.bccm.belspo.be). Deeplex-MycTB demonstrated the accurate proportion of intended susceptible and resistant populations of all mixtures above 1% (Deeplex-MycTB cut-off). All mixtures were phenotypically resistant to moxifloxacin and levofloxacin, including the mixture containing only 0.5% of *gyrA* D94G bacilli. Our results demonstrate that the Xpert MTB/XDR assay detects resistance to fluoroquinolones when *gyrA* D94G variants are present at ≥20%, highlighting its value as a rapid molecular diagnostic. Future studies should assess the assay's detection limit for other common *gyrA* and *gyrB* mutants, including variants that confer low-level fluoroquinolone resistance.

P28

The microbiological confirmation of leprosy patients with tongue swabs exhibits a lower sensitivity compared to nasal swabs

L Krausser [1 2 3] A Said [4 5] N Attoumane [4 5] S H Grillone [4 5] M Van Dyck-Lippens [2] A Baco [4 5] W Abdou [4 5] A Mzembaba [4 5] E Hasker [2] Y Asoumani [4 5] B C de Jong [2] S M Braet [1 2 3]
1: University of Antwerp, Belgium 2: Institute of Tropical Medicine, Antwerp, Belgium 3: Research Foundation Flanders (FWO), Brussels, Belgium 4: Damien Foundation, Brussels, Belgium 5: National Tuberculosis and Leprosy Control Program, Moroni, Comoros

Skin biopsies remain the most reliable source for *Mycobacterium leprae* bacilli to microbiologically confirm leprosy patients. In an effort to find less invasive sampling techniques, we compared nasal swabs and tongue swabs obtained from clinically confirmed leprosy patients enrolled in the PEOPLE study in the Comoros.

Twenty-three patients were requested to provide a swab sample from the anterior two-thirds of the tongue dorsum, along with a nasal swab and a biopsy from the margin of a skin lesion. DNA extracted from these concurrent samples was quantified with the *M. leprae* specific RLEP qPCR assay. In addition, all swab-derived DNA extracts were checked for human mitochondrial DNA to confirm adequate sampling.

M. leprae DNA was detected on 8/23 (34,8 %) tongue swabs, 12/23 (52,2 %) nasal swabs and in 16/23 (69,6 %) skin biopsies. Although the Cq values from the various sampling sites correlated, the tongue swab extracts had an average delay of nine cycles compared to the corresponding nasal swabs, suggesting that the burden of *M. leprae* on the tongue is lower than in the nasal cavity. Only multibacillary patients tested positive for *M. leprae* on tongue swabs.

This is the first study to detect *M. leprae* on the tongue dorsum in a third of leprosy patients, although DNA yield is considerably lower than for nasal swabs and skin biopsies. We will continue to optimise the tongue swab sampling to increase the confirmation rate of clinically diagnosed leprosy patients.

P29

Validation and implementation of thin-layer agar for direct *Mycobacterium tuberculosis* testing for bedaquiline resistance: a promising technique to increase access in low-resource settings.

I Cuella Martin [1] D Runyambo [2] B E Niyigena [2] S De Bock [1] M Diels [1] E Ardizzoni [1] B C de Jong [1] J C.S Ngabonziza [1 3 4] L Rigouts [1 5]
1: Unit of Mycobacteriology, Institute of Tropical Medicine, Antwerp, Belgium 2: National Reference Laboratory Division, Department of Biomedical Services, Rwanda Biomedical Center, Kigali, Rwanda 3: Research Innovation and Data Science Division, Rwanda Biomedical Centre, Kigali, Rwanda 4: Department of Clinical Biology, University of Rwanda, Kigali, Rwanda 5: Department of Biomedical Sciences, Antwerp University, Antwerp, Belgium

Since 2018, WHO recommends bedaquiline (BDQ) for treatment of rifampicin-resistant tuberculosis (RR-TB), yet the correlation between mutations and phenotypic resistance is incompletely understood, hindering molecular testing. Moreover, the great variety in level of BDQ resistance mutations seen complicates design of a rapid molecular assay. Thus, phenotypic tests (pDST) remain indispensable, but require BSL3 laboratories and have a long turnaround time, so few patients who start BDQ access such tests.

We validated thin-layer agar (TLA), for determining the minimal inhibitory concentration (MIC) of BDQ in isolates (indirect DST, 0.008-2.0 µg/ml), as well as its use for primary isolation of *M.tuberculosis* with direct DST to BDQ, rifampicin, isoniazid and levofloxacin at the National Reference Laboratory in Rwanda.

Results of indirect DST on 21 clinical isolates and 10 in-vitro resistant strains, compared with parallel 7H11 standard testing, showed a higher accuracy when interpreted on day 7 vs day 14 (94.0% vs 84.7%), and with plate incubation in standard incubator compared to incubation at 5-10% $C0_2$ (96.4% vs 81.9%). When used as direct method on 104 RR-TB decontaminated fresh samples, primary isolation was successful in 47 patients vs 57 in solid culture. However, only 3 (2.9%) TLA plates vs 7 (6.7%) solid culture were contaminated. DST results for RIF and IHN were

75% concordant with solid culture,yet TLA had a quicker turnaround time: median 19 days (IQR,14-21). On 41 patients with valid BDQ direct DST results, all were BDQ susceptible. These results confirm that TLA is a fast and reliable technique for the pDST of BDQ.

P30

Prospective evaluation of targeted next-generation sequencing of *Mycobacterium tuberculosis* complex strains in routine diagnostics in Germany.

D Nadarajan [1] P Beckert [2] M Kuhns [1] C Utpatel [2] I Barilar [2] V Dreyer [2 3] I Friesen [1] S Niemann [2 3] F P Maurer [1 4]
1: National and Supranational Reference Laboratory for Mycobacteria, Research Center Borstel, Leibniz Lung Center, Borstel, Germany 2: Molecular and Experimental Mycobacteriology, Research Center Borstel, Borstel, Germany 3: German Center for Infection Research (DZIF), Partner Site Hamburg-Lübeck-Borstel-Riems, Borstel, Germany 4: Institute of Medical Microbiology, Virology and Hygiene, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

To provide effective tuberculosis (TB) treatment, rapid diagnostics and drug susceptibility testing (DST) is crucial. Targeted next-generation sequencing (tNGS) from primary patient material can bridge the gap between cultural DST and nucleic acid amplification tests. In this study, six commercial and published DNA extraction and purification methods (BRUKER GenoLyse and FluoroLyse, BD MAX MDR-TB, QIAGEN QIAamp DNA Micro Kit, Molbio Trueprep AUTO and a published protocol (George et al. 2020)) from primary patient material were evaluated. Extracted DNAs were prescreened for TB, positive samples were subjected to tNGS (Deeplex Myc-TB, GenoScreen) and sequenced. All tested DNA extraction methods led to acceptable tNGS success rate, differences in the quality and quantity of DNA obtained was observed. For prospective evaluation we analyzed 1231 (354 sputum and 877 non-sputum) patient samples between May 2021 and December 2022 using the Molbio Trueprep AUTO DNA extraction and MTB Plus PCR assay. In total, 90 (41 sputum and 49 non-sputum; 36 smear positive, 50 smear negative, 4 no data) TB positive samples were further analyzed with tNGS. Full tNGS DST profiles of Molbio MTB Plus positive samples were obtained for 29, insufficient for 6, failed for 37 and 18 samples were excluded prior to sequencing. tNGS from primary patient material can be included in routine TB diagnostics and provides comprehensive DST data prior to culture positivity. However, the implementation of tNGS, especially in low resource countries, is impaired by the comparably high cost per sample, the need for expensive sequencing equipment and highly trained personnel.

P31

Validation of Capilia TB-Neo for identifying *M. tuberculosis* complex in culture isolates

S Chung [1] J BX Tan [1]
1: Singapore General Hospital

Tuberculosis is the second leading cause of death due to infectious disease globally, with an estimated 10 million people developing the disease annually. Central Tuberculosis Laboratory currently uses MALDI-TOF mass spectrometry and MGIT TBc identification test (TBcID) to detect *M. tuberculosis* complex (MTC) in solid and liquid media respectively. As the Capilia TB-Neo can be used to detect MTC in solid and liquid media, we evaluated its performance with the aim of streamlining our workflow.

Among 15 solid cultures, Capilia TB-Neo was positive in 7 MTC cultures and negative in 8 nontuberculous mycobacteria (NTM) cultures that were identified by MALDI-TOF. Among 33 liquid cultures, Capilia TB-Neo was positive in 17 MTC cultures and negative in 14 NTM cultures that were identified using TBcID. There were 2 MTC liquid cultures that were negative on TBcID and Capilia TB-Neo - these were subsequently identified as *M. bovis* BCG by the GenoType MTBC test. Out of 10 non-mycobacterial liquid cultures, 7 were negative on Capilia TB-Neo and weak positive on TBcID, whereas 3 were positive on both Capilia TB-Neo and TBcID.

The Capilia TB-Neo had an overall sensitivity of 92.3% (24/26), specificity of 90.6% (29/32), PPV of 88.9% (24/27) and NPV of 93.5% (29/31). Capilia TB-Neo can be considered as an alternative test for identifying MTC in solid and liquid media as it is a simpler and less time-consuming procedure compared to the MALDI-TOF in addition to being non-inferior to the TBcID.

P32

Mechanical lysis is critical to ensure optimal yield of DNA from mycobacterial cells

R de Zwaan [1] A Mulder [1] M Kamst [1] D van Soolingen [1] R M Anthony [1]
1: National Institute for Public Health and the Environment

Since 2019 the Tuberculosis Reference Laboratory in the Netherlands has routinely applied whole genome sequencing (WGS) on positive *M. tuberculosis* complex cultures. The DNA isolation was done on heat inactivated positive cultures (20 min at 80°C) using the commercial QIAamp DNA mini kit from Qiagen. This resulted in low DNA concentrations, to increase the yield we added a bead beating step to improve the cell lysis. To determine the impact of the mechanical lysis we compared results of four months before and after the introduction of bead beating. During the first four months 186 diagnostic isolates where processed compared to 237 isolates in the second four months. The average DNA concentration went from 2 ng/µl without bead beating to 94 ng/µl with bead beating. The effect on average WGS coverage was less significant (average coverage from 116 vs 136). Most importantly the WGS success rate (coverage >50) went from 87% to 95%. Eight percent of the pure cultures (mapped reads >0.88) prior to the introduction of bead beating failed compared to two percent of the isolates after the introduction of bead

beating. Bead beating of heat killed cells directly prior to DNA isolation increases the DNA concentration greater than tenfold and improved the WGS success rate.

P33

Evaluation of a new rapid kit for MALDI-TOF MS Mycobacteria identification

A Camaggi [1] M Casagrande [1] M S Caroppo [1] S Andreoni [1]
1: AOU Maggiore della Carità, Laboratory of Microbiology and Virology – Novara, Italy

In recent decades, non-tuberculous mycobacteria (NTM) infection rates raised, due to the increased number of immunocompromised individuals. Fast and accurate identification tools are essential for an effective an appropriate treatment of NTM infections. MALDI-TOF MS has become a reliable tool for mycobacteria identification, despite their challenging cell wall structure. Here, we evaluated the new MTB Mycobacteria-IVD kit (Bruker Daltonics, Germany), based on chemical cell-inactivation and mechanical-disruption of cell aggregates, using Line Probe Assay (GenoType Mycobacterium CM/AS-Hain-Lifescience,GmbH) and/or DNA sequencing results as a reference.

Fifty various NTM species were cultured on solid and liquid media and on blood cultures bottles. A mycobacterial biomass was washed and inactivated with the ready-to-use reagents (Washing Solution, Inactivation Reagent) for 30 minutes at room temperature. After centrifugation (2 min), the pellet was mixed with formic acid and acetonitrile, vortexed and centrifuged at max-speed for 2 minutes. Supernatant (1 µL) was spotted in triplicate onto the MALDI plate. Once dried, Bruker Matrix-HCCA was added, and MALDI-TOF MS identification was performed, using Mycobacteria IVD Library v2.0. Overall, the identification was achieved in less on 45 minutes. Results interpretation was performed according to the manufacturer's instructions: log(score) ≥ 1.80 highly-probable identification, log(score) between 1.60 and 1.79 probable identification and log(score) <1.60 not assigned identification. Identifications were correct for all NTMs examined. From liquid media culture (low biomass) identifications showed a log(score)< 1.60 but correct.

Our results suggest that Mycobacteria-IVD kit could provide a great advantage for timely and cost-saving NTMs identification with potential relevant implications for patient outcome.

P34

Evaluation of a new molecular assay for tuberculous and nontuberculous mycobacteria rapid detection in clinical samples

A Camaggi [1] M Mantovani [1] M S Caroppo [1] S Andreoni
1: AOU Maggiore della Carità, Laboratory of Microbiology and Virology – Novara, Italy

The introduction of molecular diagnostic assays has contributed to better control of tuberculous (TB). Since 2013, WHO recommended rapid molecular tests as the initial TB diagnostic test. In the last years, nontuberculous mycobacteria (NTM) have emerged as important human pathogens

too. In tuberculous low incidence countries, the rapid detection of NTM versus *Mycobacterium tuberculosis* complex (MTB) are useful for the management of mycobacterial diseases, because many NTM strains are resistant to the antibiotics used for TB treatment. In this study, we evaluated the performance of a novel real-time PCR cartridge-based kit, the STANDARD M10 MTB/NTM assay (SD Biosensor, Suwon, South Korea-M10), for the detection of MTB and NTM, in pulmonary and extrapulmonary samples in less than two hours. The results obtained with M10 were compared with the microscopy (AFB), culture and Xpert MTB/RIF Ultra (ULTRA) results. In this study, 37 several specimen types (sputum, bronchial wash samples, abscesses, cerebrospinal fluid, urine) collected in our Clinical Laboratory were investigated. M10 and ULTRA MTB results were both the same, except for one cerebrospinal fluid (trace ULTRA and M10 negative) and one urine samples (low ULTRA and negative M10 result but MTB culture negative). NTM results were positive for all microscopy positive specimens whereas only 4 of the 12 negative AFB samples but NTM positive culture samples were detected by M10. These preliminary data suggest that STANDARD M10 MTB/NTM could be included in the clinical microbiology laboratories workflow of countries with low incidence of tuberculous that daily searches for MTB and NTM.

P35

Xpert MTB/XDR assay for the rapid diagnosis of TB resistance. A country wide cross sectional observational prospective study from Pakistan

F Saluzzo [1] F Masood [2] V Batignani [1] A Hussein [2] D M Cirillo [1] S Tahseen [2]
1: San Raffael Scientific Institute 2: National TB Reference Laboratory, National Tuberculosis Control Program Pakistan

Pakistan is among top five high Drug Resistant Tuberculosis (TB) countries. Innovative tools for the rapid detection of isoniazid and fluoroquinolones resistance in this setting may play a pivotal role in increasing the rate of early appropriate treatment, finally reducing the disease burden.

In this cross sectional observational prospective study, clinical samples from 23 different sites among Pakistan have been tested to evaluate the performance of Xpert MTB/XDR assay and if Xpert MTB/XDR assay (XDR) could replace Line Probe Assay (LPA) in routine settings.

A total of 525 clinical samples were collected from people recently with TB regardless of rifampicin resistance result. XDR, LPA and phenotypic Drug Susceptibility Test (pDST) have been performed. Strains with discordant results for isoniazid and fluoroquinolones resistance at XDR and pDST have been analysed with Whole Genome Sequencing (WGS) and/or targeted Next Generation Sequencing (tNGS).

Xpert MTB/XDR provided interpretable results in 95,8% of all the analysed samples, while LPA provided an interpretable result in less than 80% of the analysed strains.

The overall sensitivity and specificity of XDR were 90,3 % (CI 95% 87,7-92,4%) and 98.5% (CI 95% 97,1-99,2%). WGS and tNGS outputs analysed according to the WHO catalogue confirmed XDR result in 3/11 discordant results for isoniazid, in 1/11 a frameshift mutation was detected confirming pDST result. In 4/11 cases mutations of uncertain significance were identified in strains resulted resistant at pDST and sensitive at XDR.

In conclusion, XDR proved to be a reliable tool for the rapid diagnosis of isoniazid and fluoroquinolone resistance.

P36

The PEOPLE trial on post exposure prophylaxis for leprosy

E Hasker [1] Y Assoumani [2 8] A Randrianantoandro [3] S Ramboarina [4] S Braet [1] B Cauchoix [4] A Baco [2 8] A Mzembaba [2] Z Salim [2] M Amidy [2] S Grillone [2] N Attoumani [2] S Grillone [2] M Ronse [1] K Peeters [1] H Harinjatovo [3] P Supply [5] R Snijders [1] C Hoof [1] P Suffys [6] T Rasamoelina [7] N Ortuno-Gutierrez [8] A Geluk [9] E Cambau [10] B C de Jong [1]

1: Institute of Tropical Medicine, Antwerp 2: National Tuberculosis and Leprosy control Program, The Union of Comoros 3: National Leprosy control Program Madagascar 4: Fondation Raoul Follereau, Antananarivo, Madagascar 5: Genoscreen SAS, Lille, France 6: Oswaldo Cruz Institute, Fiocruz, Laboratory of Molecular Biology Applied to Mycobacteria, Rio de Janeiro, Brazil 7: Centre d'Infectiologie Charles Mérieux, Antananarivo, Madagascar 8: Damien Foundation, Brussels, Belgium 9: Leiden University Medical Center, Leiden, the Netherlands 10: Assistance publique-Hopitaux de Paris, Paris, France

Introduction: Post-exposure prophylaxis with single dose rifampicin (SDR-PEP) can block progression from infection with *M.leprae* to leprosy disease. In the PEOPLE trial we assessed effectiveness of different modalities of SDR-PEP.

Methods: We randomized 64 villages (total population 110,660) in Comoros and Madagascar to four study arms. In each arm we conducted four rounds of annual door-to-door screening and treated all leprosy patients identified. In arm 1 nothing else was done, in arms 2-4 we also provided SDR-PEP to all household contacts. In arm 3 SDR-PEP provision was extended to anyone living within 100 meters of an index case, in arm 4 SDR-PEP was also offered to anyone living within 100 meters and testing positive to anti-PGL-I. We compiled incidence rate ratios (IRR) of leprosy between the comparator arm (arm 1) and each of the intervention arms and explored spatial associations.

Results: We found some reduction in incidence in arms 3 (IRR 0.8) and 4 (IRR 0.6) but differences were not statistically significant. Controlling for baseline prevalence there was a borderline significant reduction in arm 3, IRR 0.5 (98.3% CI 0.3-1.0). At individual level SDR-PEP was protective (IRR 0.6, 95% CI 0.4-0.9). Risk of leprosy was two to four times higher for those living up to 75 meters of an index case.

Discussion/conclusion: SDR-PEP appears to protect but less than expected. We found strong spatial associations up to 75 meters from index cases. Most impact on transmission can be expected from well targeted door-to-door screening, to which SDR-PEP can add.

P37

Serratia sp. clusters in bronchoalveolar lavage specimens from patients with tuberculosis and non-tuberculous *mycobacterial lung diseases*

M Belheouane [1] B Kalsdorf [2] J Heyckendorf [2 3] S Niemann [4] K I Gaede [5 6] M Merker [1]
1: Evolution of the Resistome, Research Center Borstel, Borstel, Germany 2: Clinical Infectious Diseases, Research Center Borstel, Borstel, Germany 3: Department of Internal Medicine I, University Medical Center Schleswig-Holstein, Kiel, Germany 4: Molecular and Experimental Mycobacteriology, Research Center Borstel, Borstel, Germany 5: BioMaterialBank Nord, Research Center Borstel, Borstel, Germany 6: German Center for Lung Research (DZL) Airway Research Center North (ARCN)

Bronchoalveolar lavage fluid (BALF) remains a crucial central diagnostic tool in tuberculosis (TB), and non-tuberculous mycobacterial (NTM) lung diseases. Here, we investigated 66 BALF specimens, sampled from TB and NTM patients. We depleted human cells and extracellular DNA, and profiled the microbiome using 16S rRNA amplicon sequencing following state-of-the-art laboratory, and analytical protocols optimized for low biomass specimens.
First results indicate that distinctive clusters of *Serratia sp.* are associated with TB and NTM patients. Importantly, *Serratia* is considered a significant opportunistic pathogen, which has been showing a strong increase of multi-drug resistance. Moreover, using quantitative assays, and the Deeplex® Myc-TB technology, we plan to define *Mycobacteria* loads, and the resistance profiles of infectious pathogens.

We will integrate the characteristics of the pathogens, and the entire lung microbial communities to search for microbial signatures which likely contribute to disease severity, and therapy success; eventually paving the way for the inclusion of lung microbiome in designing future personalized treatments in lung infectious diseases.

Keywords: Bronchoalveolar lavage fluids, *Mycobacterium*, *Serratia*, lung microbiome.

P38

Rifampicin and isoniazid dosage adjustment according to TDM and acetylator status: a single centre prospective observational study

M Schiuma [1] A Torre [2] A Civati [1] A Jacobs [3] A Giacomelli [1 2] S Antinori [1 2]
1: Department of Biomedical & Clinical Sciences "Luigi Sacco", Università degli Studi di Milano, Italy. 2: III Infectious Diseases Unit, ASST Fatebenefratelli Sacco, Milano, Italy. 3: University of Cape Town, Cape Town, South Africa

Individualized dosing of rifampicin and isoniazid may improve treatment outcomes. We describe how rifampicin therapeutic drug monitoring (TDM) and N-acetyltransferase 2 (NAT2) assessment could affect drug dosage in patients with tuberculosis (TB) disease/infection.

A prospective observational study enrolling consecutive subjects managed at Sacco Hospital (Italy) for TB disease/infection (July 2020-April 2023) was performed. Rifampicin TDM levels (range 8-24 mg/L) were systematically determined (mass spectrometry) within two weeks from enrollment at 2, 4 and 6 hours after intake. A reference area under the curve (AUC) of 67.5 mg*h/L was assumed. NAT2 acetylator status was determined (RT-PCR) at enrollment and defined as slow, intermediate or rapid.

100 rifampicin TDM and 120 acetylator statuses were determined in 129 subjects. 51.9% (n=67) were males, median age 49 (IQR 37-63), 79% (n=102) had TB disease. Median rifampicin dose administered was 9.93 mg/kg (IQR 9.02-11.15). Median AUC was 57.73 mg*h/L (IQR 42.58-82.98). In 70% (n=70) of cases rifampicin concentration was > 8 mg/L, in 40% (n=40) the AUC was > 67.5 mg*h/L. Rifampicin dosage was modified in 42% (n=42) cases: increased in 92.9% (n=39), reduced in 7.1% (n=3).
10 patients were rapid, 65 intermediate, 45 slow acetylators. Isoniazid dosage was modified in 21.7% (n=26) cases: increased to 7.5 mg/kg in 90% of rapid acetylators (n=9), reduced to 2.5 mg/kg in slow acetylators with hepatotoxicity (26.1%, n=17).

Rifampicin TDM and/or genotyping led to modification of drug dosage in 47.3% (n=61) cases. TDM and pharmacogenetics could guide TB treatment individualization. Further studies are required to determine its effect on hard clinical outcomes.

P39

Search for conserved sites in *Mycobacterium tuberculosis* DNA gyrase

D Zygala-Pytlos [1 2] A Minias [1] A Brzostek [1] J Dziadek [1]
1: Laboratory of Genetics and Physiology of Mycobacterium, Institute of Medical Biology, Polish Academy of Sciences, Lodz, Poland 2: The Bio-Med-Chem Doctoral School of the University of Lodz and Lodz Institutes of the Polish Academy of Sciences, University of Lodz, Lodz, Poland

DNA gyrase is an enzyme necessary for the proper functioning of *Mycobacterium tuberculosis (Mtb)*. It is the target of action of quinolones, a group of drugs used in the treatment of tuberculosis. Unfortunately, emerging mutations in the genes encoding subunits of this protein contribute to drug resistance and ineffectiveness of these compounds. The aim of the study is to search for conserved sites in DNA gyrase. These sites could serve as binding sites for new antitubercular compounds, showing greater effectiveness due to the lack of possibility of mutations at the binding site and hence restraining the development of drug resistance. On the basis of bioinformatic analyses, we selected the codons of the *gyrA* and *gyrB* genes under strong purifying selection. We chose amino acid substitutions in codons that did not cause deleterious changes in the protein based on the PredictSNP tool prediction. We genetically modified *Mtb* introducing selected point mutations into the genome. We selected 30 codons for testing. We obtained 18 mutants with point mutations, while in 12 cases, it was not possible to obtain a strain with a mutation in the selected codon. Conservative sites will be confirmed with a second attempt to mutate the site. We found codons that may be highly conserved in *Mtb* gyrase. These results require further verification. We also found that the majority of sites estimated to be under strong purifying selection by mathematical models are not hyper-conserved. The observable lack of substitutions at these sites in bacterial population remains to be explained.

P40

Single nucleotide variation catalogue from clinical isolates mapped on tertiary and quaternary structures of ESX-1 related proteins reveals critical regions as putative Mtb therapeutic targets

O Tzfadia [1 5] A Siroy [2] A Vujkovic [1 5] J Snobre [1 6] A Gijsbers [2] R Vargas [4] W Mulders [1] C Meehan [1 3] M Farhat [4] P Peters [2] B C de Jong [1 5] R Ravelli
1: Institute for Tropical Medicine 2: Maastricht University 3: Nottingham Trent University 4: Harvard Medical School 5: University of Antwerp 6: Vrije Universiteit Brussel (VUB)

Proteins encoded by the ESX-1 related genes are essential for full virulence in all *Mycobacterium tuberculosis* complex (MTBc) lineages, the pathogens with the highest mortality worldwide. Identifying critical regions in these ESX-1 related proteins could provide preventive or therapeutic targets for MTB infection, the game changer needed for tuberculosis control. We analysed a compendium of clinical MTB isolates from all lineages from >32,000 patients and identified single nucleotide variations (SNV) in ESX-1 genes. When mutations were mapped on the surface of known and alphafold-predicted ternary protein structures, fully conserved regions emerged which sat at the interface of known quaternary structures or could be predicted to be involved in yet-to-be-discovered interactions. Some clonal prevalent mutants circulated (found in >1% of the isolates): these were mostly located at the surface of globular domains, remote from known intra- and inter-molecular protein–protein interactions. Fully conserved intrinsically disordered regions (IDRs) of proteins were found, suggesting that these are crucial for the pathogenicity of the MTB. Altogether, our findings provide an evolutionary structural perspective on MTB virulence and highlight hyper conserved regions of proteins as attractive vaccine antigens and drug targets. Extending this approach to all MTB genes and other pathogens can provide a novel critical resource for the development of innovative tools for pathogen control.

P46

In Vitro Activity of Tedizolid and Omadacycline in Nontuberculous Mycobacteria

S Turkkal [1] N E Ozcatalkaya [1] E Ciftci [1] G Yaman [2] D Satana [3]
1: Istanbul University, Institute of Health Science, Department of Medical Microbiology 2: Maltepe University, Faculty of Medicine, Department of Medical Microbiology 3: Istanbul University, Istanbul Faculty of Medicine, Department of Medical Microbiology

Nontuberculous mycobacteria (NTM) are opportunistic pathogens found everywhere, especially in soil and water sources. In order to establish appropriate drug regimens against NTM infections, antibiotic susceptibility tests must be performed. In this study, the antimicrobial susceptibilities of a total of 105 NTM strains, including 64 rapid-growing and 41 slow-growing strains, to tedizolid and omadacycline were investigated using the colorimetric microdilution method according to CLSI M24 and M62 guidelines. The MIC range of 0.015-32 µg/ml was used for tedizolid and 0.003-64 µg/ml for omadacycline. Based on literature information, the critical concentration values recommended for linezolid in CLSI M62 (≤8 µg/ml susceptible; 16 µg/ml intermediate; ≥32 µg/ml resistant) were used to evaluate the susceptibility of tedizolid. Only one rapid-growing

strain, *M. fortuitum*, was found to be intermediate (1/29; 16 µg/ml) to tedizolid, and one slow-growing strain, *M. avium*, was found to be resistant (1/6; ≥32 µg/ml), while all other NTM strains were susceptible (103/105; ≤8 µg/ml). As there is no recommended critical concentration value for omadacycline, only the MIC values obtained were reported for this antibiotic. The obtained MIC values are shown in the attached table. This study demonstrates that tedizolid has a strong in vitro effect on NTM, but omadacycline gives variable results on NTM strains, indicating that species-specific antimicrobial susceptibility testing is necessary to establish NTM treatment regimens.

Table. MIC values of nontuberculous mycobacteria against tedizolid and omadacycline.

	Strains	N	MIC values against tedizolid (µg/mL)											
			≥32	16	8	4	2	1	0.5	0.25	0.125	0.06	0.03	≤0.015
RAPID GROWING NTM	*M. fortuitum*	29		1		2	1	2	10	6	3	3	1	
	M. abscessus	21						1	5	7	4	2		2
	M. simiae	4					2			2				
	M. chelonae	3					1			2				
	M. peregrinium	3						1	1	1				
	M. elephantis	1								1				
	M. neoaurum	1									1			
	M. farcinogenes	1									1			
	M. tokaiense	1								1				
SLOW GROWING NTM	*M. lentiflavum*	15						3	4	1	2	1	3	1
	M. gordonae	9								1	2	4	1	1
	M. avium	6	1					1		2		1		1
	M.intracellulare	4					1	2			1			
	M. chimera	4				1			1	2				
	M. kansasii	3							2	1				

	Strains	N	MIC values against omadacycline (µg/mL)										
			≥64	32	16	8	4	2	1	0.5	0.25	0.125	0.06	≤0.03
RAPID GROWING NTM	*M. fortuitum*	29		3	1	1	3	1	5	2	5	7	1	
	M. abscessus	21		1			6	1	1	5	3	2	1	1
	M. simiae	4	2				1		1					
	M. chelonae	3		2	1									
	M. peregrinium	3									2	1		
	M. elephantis	1							1					
	M. neoaurum	1											1	
	M. farcinogenes	1							1					
	M. tokaiense	1						1						
SLOW GROWING NTM	*M. lentiflavum*	15	3		1	1	4		2	1		1		2
	M. gordonae	9			1	1	1	2	1	2			1	
	M. avium	6	2									2		2
	M.intracellulare	4	2						1				1	
	M. chimera	4		1		1	1	1						
	M. kansasii	3	2					1						

P47

Research of In Vitro Activity of Bedaquiline In Nontuberculous Mycobacteria

N E Ozcatalkaya [1] S Turkkal [1] E Ciftci [1] G Yaman [3] D Satana [2]
1: Istanbul University, Institute of Health Science, Department of Medical Microbiology 2: Istanbul University, Istanbul Faculty of Medicine, Department of Medical Microbiology 3: Maltepe University, Faculty of Medicine, Department of Medical Microbiology

Nontuberculous mycobacterial infections caused by Mycobacterium species are increasing worldwide day by day. Despite being a treatable disease, treatment regimens can be prolonged and sometimes fail due to the usage of multiple drugs, natural or acquired resistance, and the inability to differentiate from the *Mycobacterium tuberculosis* complex. Due to the potential for resistance that may be encountered due to these problems, the discovery of new drugs is of utmost importance. It has been shown in previous studies that bedaquiline, discovered by Koen Andries and colleagues in 2005, is effective against multidrug-resistant *M. tuberculosis* and nontuberculous mycobacterial strains. Our study aimed to investigate the effect of bedaquiline, which has not yet been introduced in our country, on nontuberculous mycobacterial strains isolated from samples sent for routine examination to the Istanbul University Istanbul Faculty of Medicine Mycobacteriology laboratory and Istanbul Duzen Laboratory. The study included 62 rapid and 41 slow strains from 14 different species. As stated in the guidelines, the microdilution method was applied with a MIC range of 0.008-16 µg/ml. It was determined that all slow-growing strains may be sensitive to bedaquiline (MIC ≤0.25µg/ml), while 16 of the rapid-growing strains may be resistant (MIC >0.25µg/ml). Of the potentially resistant strains, seven were identified as *M. fortuitum*, five as *M. abscessus*, three as *M. chelonae*, and one as *M. elephantis*. In conclusion, it was observed that bedaquiline has a high activity against nontuberculous mycobacterial strains.

Table. MIC values of nontuberculous mycobacteria against bedaquiline.

	Strains	Number	MIC values against bedaquiline (µg/mL)											
			≥16	8	4	2	1	0.5	0.25	0.125	0.06	0.03	0.015	≤0.008
RAPID GROWING NTM	*M. fortuitum*	28	7											21
	M. abscessus	21	5						1		4		2	9
	M. simiae	4												4
	M. chelonae	3	3											
	M. peregrinium	3												3
	M. elephantis	1			1									
	M. neoaurum	1												1
	M. farcinogenes	1												1
SLOW GROWING NTM	*M. lentiflavum*	16												16
	M. gordonae	8												8
	M. avium	6												6
	M.intracellulare	4												4
	M. chimera	4												4
	M. kansasii	3												3

P48

Mouse models to study host-pathogens interaction in *Mycobacterium abscessus* lung infections.

F Saliu [1] N Francesca [1] D Cirillo [1] N Lorè [1]
1: San Raffaele Scientific Institute

Mycobacterium abscessus is a non-tuberculous mycobacterium (NTM) able to cause progressive pulmonary infections, named NTM pulmonary disease. The therapeutic approaches are limited, and infections are difficult to treat due to antibiotic resistance conferred by an impermeable cell wall, drug efflux pumps, or drug-modifying enzymes. The development of new therapeutics, intended as antimicrobials or drug limiting immunopathology, is urgently necessary. We recently set up a preclinical murine models of *M. abscessus* representing an useful tool to validate and translate in vitro-proofed concepts. In addition to acute model of lung infection, we refined an agar beads mouse model to establish *M. abscessus* chronic infection in the airway of immunocompetent mice. In this model, *M. abscessus* is able to establish a persistent lung infection for more than one month with a stable bacterial load. Thanks to these models, it is possible to monitor both pulmonary mycobacterial burdens and inflammatory responses occurring during the development of acute or chronic respiratory infection. The exploitation of this model is allowing us to prove its relevance for the identification of novel therapeutic targets fighting *M. abscessus* infection and its immunopathological consequence. In conclusion, the developed and characterized murine models are useful for future mechanistic studies in the field of *M. abscessus*-host interaction.

P49

New method for fast and direct Mycobacteria identification from positive blood culture

A Camaggi [1] S Tonello [2] R Minisini [2] M Mantovani [1] M S Caroppo [1] S Andreoni [1]
1: AOU Maggiore della Carità, Laboratory of Microbiology and Virology - Novara, Italy 2: Università del Piemonte Orientale, DIMET – Novara, Italy

Disseminated nontuberculous mycobacterial (NTM) infection is an infectious disease that occurs mostly in immunocompromised hosts. The use of a fully automated method, as BACTEC-MYCO/F-LYTIC (Becton Dickinson Sparks, MD, USA) bottles system, represents a sensitive method for mycobacteremia detection. Unfortunately, Line Probe Assay or other conventional commercial assays for mycobacteria identification could not be used for samples containing blood (subculture on liquid or solid media are required before). In this study we evaluate the MBT-Sepsityper Kit (Bruker Daltonik, GmbH, Germany) for the direct mycobacteria identification from positive blood cultures bottles (BCBs) using MALDI-TOF MS.

Seventy MYCO/F-LYTIC-bottles were inoculated with human blood (4.9 mL) and various NTM species suspension (0.1 mL at 0.5 McF): *M. abscessus*, *M. avium*, *M. chelonae*, *M. chimaera*, *M. elephantis*, *M. fortuitum*, M. *hassiacum*, *M. intracellulare*, *M. llatzerense*, *M. lentiflavum*, *M. mucogenicum*, *M. nebraskense*, *M. parascrofulaceum*, *M. simiae*. Once flagged positive and AFB documented, 5.0 mL of blood culture were centrifugated and 1.0 mL of pellet underwent Sepsityper preparation, following the manufacturer's instructions (classical protocol) and our

homemade inactivation/extraction protein steps (ethanol/formic acid-acetonitrile) for MALDI mycobacteria identification. All the samples were correctly identified at the species level except *M. chimaera* and *M. intracellulare*. Their mass spectra are more similar and by standard MALDI-TOF library they couldn't be differentiated.

These data show how the employment of the Sepsityper-kit for the mycobacteria identification with MALDI-TOF in BCBs allows a rapid (~2 hours) and reliable identification leading to a significant decrease in timing to results (5-15 days depending on mycobacteria species).

P50

Identification and specie-typing of nontuberculous mycobacteria among sputum samples of presumed and diagnosed drug-resistant tuberculosis patients in Ghana, a 10-year retrospective laboratory analysis

E T Abbew [1 2 3] R Laryea [4] F Sorvor [5] Y Aduse-Poku [5] N Lorent [6] T Decroo [1] D Obiri-Yeboah [7] L Lynen [1] L Rigouts [1 2]
1: Institute of Tropical Medicine, Antwerp 2: University of Antwerp 3: Cape Coast Teaching Hospital, Ghana 4: Eastern Regional Hospital, Ghana 5: National Tuberculosis Control Program Ghana 6: University Hospitals, Leuven 7: University of Cape Coast, Ghana

The awareness of nontuberculous mycobacterial pulmonary disease (NTM-PD) is rising worldwide with the increasing isolation of NTM from sputum samples. In 2009, Ghana started using mycobacteria growth indicator tube (MGIT) for diagnosing and monitoring drug-resistant tuberculosis (DR-TB) patients, also resulting in increasing isolation of NTM species.

We retrospectively analysed existing laboratory data from 2012-2021 from the Eastern Regional Hospital (ERH) and Cape Coast Teaching Hospital (CCTH). All sputum samples had microscopy and culture done using MGIT and solid medium. All probable NTM isolates were stored at -20°C. After regrowing them in MGIT they were characterised using GenoType CM/AS (Bruker, Germany).

A total of 2492 sputum samples were analysed with smear and culture over the ten years, with one-third of them being culture positive (33.7%). Of these, 30.6% were classified and stored as NTM.

Overall, of 225 NTM isolates that could be regrown, 15.1% were *M. intracellulare*, 4.9% were *M. fortiutum*, 0.9% were *M. abscessus*, 0.9% were *M. malmoense*, 0.4% were *M. avium* and *M. gordonae*. A further 8% were identified as MTB complex. 37.5% isolates could not be identified; they will undergo Sanger sequencing using the 16rRNA and/or *rpoB* targets. An important proportion (19.1%) remained negative by the Genotype assays.

The isolation and identification of potentially pathogenic NTM species in this TB-endemic setting suggests that incorporating NTM species identification in the routine work flow may help with clinical decision-making of NTM-PD in Ghana.

P51

Phenotypic drug susceptibility patterns of *Mycobacterium avium* complex clinical isolates in Russia

D Starkova [1] V Zhuravlev [2] N Solovieva [2]
1: St Petersburg Pasteur Institute, St Petersburg, Russia 2: St Petersburg Research Institute of Phthisiopulmonology, St Petersburg, Russia

Over the past 10 years, the number of respiratory infections caused by the *Mycobacterium avium* complex (MAC) has increased considerably in Russia. The high level of drug resistance (both intrinsic and acquired) and lack of breakpoints for many antibiotics are major issues in treating of MAC infections. The aim of this study was to investigate the phenotypic drug susceptibility of MAC strains isolated from patients with mycobacteriosis in Northwestern Russia. In total, 192 slow-growing MAC strains (164 - *M. avium*, 28 - *M. intracellulare*) isolated in 2014-2020 were studied. Of the 164 *M. avium* strains, 116 were isolated from newly diagnosed patients, 48 from previously treated patients. Drug susceptibility testing was performed using Sensititre SLOMYCO panels (Thermo Fisher Scientific, USA). For clarithromycin (CLA), moxifloxacin (MXF), linezolid (LZD) and amikacin (AMI), CLSI breakpoints have been used to interpret MIC values. Of the four antibiotics, CLA was the most effective against both *M. avium* (67.1%; 110/164) and *M. intracellulare* (60.7%; 17/28) without significant difference between species (p>0.05). Overall, 57.3% *M. avium* and 57.1% *M. intracellulare* isolates were susceptible to LZD. For MXF, 26.8% *M. avium* and 14.3% *M. intracellulare* isolates were susceptible; for AMI - 57.3% *M. avium* and 53.5% *M. intracellulare* isolates were susceptible, respectively. The proportion of CLA-susceptible *M. avium* isolates was 20% higher in newly diagnosed patients compared to previously treated patients (p=0.013). In conclusion, the macrolides are the most effective in the therapy of MAC infections, however, CLA-resistant strains emerged significantly more frequently after treatment.

P52

Is M. saskatchewanense misidentified as *M. intracellulare* using the DNA STRIP technology-based method?

F Bisognin [1 3] F Messina [2] V Ferraro [1] O Butera [2] F Sorella [3] E Girardi [2] A Cannas [2] P Dal Monte [1 3]
1: IRCCS Azienda Ospedaliero-Universitaria di Bologna 2: IRCCS National Institute for Infectious Diseases L. Spallanzani 3: Alma Mater Studiorum, University of Bologna

M. saskatchewanense is a recently identified species of pigmented and slowly growing Non-Tuberculous Mycobacteria (NTM), positive to Mycobacterium avium complex (MAC) by AccuProbe system. MAC organisms have been frequently isolated from water samples, collected from different medical devices.

This study aims to assess if DNA STRIP technology misidentified *M. saskatchewanense* with mycobacteria belonging to *Mycobacterium avium* complex.

From August 2022 to February 2023, 346 medical device water samples were processed by the referral centre for the detection of mycobacteria in the Emilia-Romagna region, Italy.

Twenty-one MGIT cultures were positive for NTM. GenoType Mycobacterium CM (CM, Bruker) was used as first step for strain identification, and all were found to be components of MAC. A further procedure with GenoType NTM-DR (NTM-DR, Bruker) was performed to discriminate among the different species. Subcultures from positive MGIT were made on Middlebrook 7H11 (BD) to obtain a microbial growth for: (i) phenotypical confirmation by MALDI-TOF mass spectrometry and (ii) molecular confirmation by WGS (MiSeq Illumina) at INMI “L. Spallanzani”, Rome.

All positive cultures were identified as *M. intracellulare* by CM and NTM-DR assays but, in contrast, colony morphology showed a bright yellow scotochromogenic growth. MALDI-TOF analyses identified the strains as *M. saskatchewanense* with a high score, and the identification were confirmed by WGS analysis. Sequences are available under the BioProject accession number: PRJNA937079.

MALDI-TOF analysis, supported by colony morphology and confirmed by WGS, identified NTM as *M. saskatchewanense* while NTM-DR, the most common genotypic methods used to discriminate strains belonging to MAC complex, misidentified it as *M. intracellulare* species.

P54

Whole genome sequencing analysis for confirmation of suspected *Mycobacterium avium* laboratory cross-contamination

S Mok [1 2] E Roycroft [1 2] P R Flanagan [1 2] M Keane [1] J Wagener [1 2] B Boyle [1 2] M M Fitzgibbon [1 2]
1: Irish Mycobacteria Reference Laboratory, St. James's Hospital 2: Trinity College Dublin

False-positive cultures caused by laboratory cross-contamination of *Mycobacterium tuberculosis* are well-documented. Such contamination events are often confirmed with molecular techniques. In contrast, laboratory cross-contamination of non-tuberculous mycobacteria (NTM) is much less documented. Here we describe the investigation of two suspected *Mycobacterium avium* cross-contamination cases in an ISO15189 accredited reference laboratory using whole genome sequencing (WGS). Cross-contamination was considered in two patients, each with a single positive-culture and with no clinical suspicion of NTM lung disease. Further investigation was performed by reviewing all laboratory processes and WGS of retrospective *M. avium* isolates that were identified in the laboratory in 2022. WGS of *M. avium* isolates (n=65) was performed using the Illumina MiSeq and the species identification was confirmed using NTM-Profiler. WGS analysis was performed using MTBseq and Snippy, and *de novo* assembly was also performed on the suspected source isolate and used as a reference genome for confirmation. Two cases of *M. avium* cross-contamination were identified and confirmed by WGS. WGS results suggested cross-contamination from a strongly positive *M. avium* culture (3+ AFB smear and time to positivity 3 days) that occurred on two separate occasions, which may have been caused by aerosol generation during laboratory testing. There were no further cases of *M. avium* cross-contamination detected. There was no impact on patient care and corrective actions were implemented in the laboratory. This study highlights the added value of WGS for NTM

identification and relatedness analysis, and the possibility of cross-contamination in a setting where NTM infections are on the rise.

P55

Host specific in vitro virulence of different *Mycobacterium tuberculosis* ecotypes

M Caballer-Gual [1] G Santamaria [1] C Gomis-Olcina [1] C Renau-Minguez [1]
A Espert-Rehues [1] P Ruiz-Rodriguez [1] M Coscolla [1]
1: I2SysBio, University of Valencia-FISABIO Joint Unit, 46980 Paterna, Spain

Tuberculosis (TB), a leading cause of adult death from infectious agents worldwide, is caused by a complex of phylogenetically related bacteria comprising various Mycobacterium tuberculosis (MTB) ecotypes. This complex consists of nine human-associated lineages and four animal-associated lineages. A deeper understanding of host-pathogen interactions in TB is essential to comprehend the co-evolution of pathogens and hosts, ultimately leading to the development of novel strategies to control MTB.

We hypothesise that virulence is influenced by host specificity, suggesting that MTB strains exhibit greater virulence in their preferred hosts, and that specific bacterial-host signatures can be identified. To test this hypothesis, we conducted experiments in which bovine and human macrophage cell lines were infected with strains from two animal-associated lineages.

Our analysis demonstrated that preferred host-ecotype combinations, as derived from epidemiological data, resulted in higher necrosis levels and more robust growth dynamics during in vitro infection compared to non-preferred bacterial-host combinations. Additionally, to describe the dynamics of bacteria and host-specific signatures during in vitro infection, we analysed and characterised bacterial and host expression signatures across the different host-pathogen combinations. This approach allowed us to uncover unique patterns and relationships between host and pathogen, providing insights into the mechanisms underlying host-specific virulence.

Further investigations incorporating metabolomic and proteomic analyses could provide additional specific molecular signatures of host-pathogen-specific virulence, advancing our understanding of the complex interactions between MTB and its hosts. This knowledge could ultimately contribute to the development of improved diagnostic tools, therapies, and preventative measures against TB.

P56

BCCM/ITM: a public repository for safe and easy accessible quality-assured mycobacterial strains and services

A Avakimyan [1] S Cogneau [1] M Diels [1] V Van de Perre [2] B C de Jong [3] L Rigouts [1 3]
1: BCCM/ITM, Institute of Tropical Medicine, Antwerp, Belgium 2: BCCM Cooridnation Cell, Belgian Federal Science Policy, Brussels, Belgium 3: Mycobacteriology Unit, Institute of Tropical Medicine, Antwerp, Belgium

The Belgian Coordinated Collections of Microorganisms (BCCM) aim to share quality-assured biological material, related information, and its know-how, to the benefit of its partners and clients in academic and industrial communities. BCCM unites 7 decentralized biological resource centers, including the Collection of Mycobacterial Cultures at the Institute of Tropical Medicine (https://bccm.belspo.be/about-us/bccm-itm).

To foster scientific advance and quality diagnostics, BCCM/ITM preserved and characterized *Mycobacterium tuberculosis* complex (MTBc) and non-tuberculous mycobacterial (NTM) strains, including a *M. bovis* BCG transposon library. These >1200 publicly available strains belong to all human- and some animal-adapted MTBc lineages and represent (combinations of) resistance to old and new anti-TB drugs. The majority of our MTBc strains have extended phenotypic drug-susceptibility (DST) or minimal inhibitory concentration (MIC) results, complemented with targeted or whole genome sequence data, all searchable in our online catalogue. Since 2018, we distributed 1084 MTBc and 149 NTM strains, and 56 BCG mutants to external clients, either as freeze-dried material, gDNA or thermolysates.

FAIR science aims at sharing scientific output to maximize the access, reuse and impact of research, by depositing biological materials in public repositories along with the datasets (sequences, etc). Public deposit at BCCM/ITM is free of charge, while safe deposit - safeguarding your biological materials at an independent location - comes with a cost.

BCCM/ITM extended its customer-oriented service portfolio, offering genotypic and phenotypic identification and DST, including MIC determination by broth microdilutions testing. We invite the scientific community to use this resource to the advantage of patients affected by mycobacterial diseases.

P57

Clusters of multidrug-resistant *Mycobacterium tuberculosis* strains from newly-diagnosed patients in Northwest Russia

A Vyazovaya [1] A Gerasimova [1] R Mudarisova [1] D Terentieva [1] N Solovieva [2] V Zhuravlev [2] I Mokrousov [1]
1: St Petersburg Pasteur Institute, St Petersburg, Russia 2: St Petersburg Research Institute of Phthisiopulmonology, St Petersburg, Russia

Multidrug resistant tuberculosis (MDR-TB) constitutes a major health problem in Russia and globally. Certain *Mycobacterium tuberculosis* genotypes are more clinically/epidemiologically significant due to the higher transmissibility and association with MDR. The aim of this study was to perform molecular characterization of the recent collection of *M. tuberculosis* MDR strains from newly-diagnosed TB patients in Northwest Russia. We studied 497 *M. tuberculosis* strains isolated in 2017-2019. The Beijing genotype and its main subtypes B0/W148, Central Asian/Russian and CAO (Central Asian Outbreak) were determined by PCR of specific markers and 24 MIRU-VNTR typing (followed by comparison with MIRU-VNTRplus.org). Non-Beijing isolates were spoligotyped and compared to the SITVIT2 database. MDR was detected in 35.2% (175) of strains and was caused mainly by mutations *rpoB* Ser450Leu and *katG* Ser315Thr. Among MDR strains, Beijing genotype strains prevailed (84.6%; 148/175): modern Beijing (n=142) and ancient/ancestral Beijing (n=6, all type 1071-32). Beijing B0/W148 strains (44.6%; 66/148) were predominantly represented by type 100-32 (74.2%; 49/66). Central Asian/Russian genotype strains (48.0%; 71/148) were more heterogeneous by VNTR loci than B0/W148 strains (HGI 0.74 vs HGI 0.45). The Central Asian/Russian strains were represented by the following clusters: 94-32 (33/71; 46.5%), 1065-32 (17/71; 23.9%), 95-32 (6/71; 8.5%). Eleven strains belonged the CAO genotype. Non-Beijing strains (15.4%; 27/175) were represented by the families LAM, Ural, T, Haarlem. Thus, the increase in the proportion of primary MDR-TB in Northwest Russia is due to the continued active transmission of *M. tuberculosis* Beijing strains, mainly those of genotype B0/W148.

Acknowledgements: Russian Science Foundation (grant 19-14-00013).

P58

Comprehensive Quality Assurance Intervention for Reliable Drug Susceptibility Testing Results for TB in Armenia

S Andres [1] I Friesen [1] E Kabasakalyan [5] N Shubladze [6] C U Köser [2] L de Araujo [3] C Utpatel [3] I Barilar [3] A Yedilbayev [4] S Niemann [1 3] S Ehsani [4]
1: National and World Health Organization Supranational Reference Laboratory for Mycobacteria, Research Center Borstel, Leibniz Lung Center, Borstel, 23845, Germany 2: Department of Genetics, University of Cambridge, Cambridge, Downing Street, Cambridge CB2 3EH, UK. 3: Molecular Mycobacteriology, Research Center Borstel - Leibniz Lung Center, Borstel, 23845, Germany 4: World Health Organization, Regional Office for Europe, Joint Infectious Diseases Programme, Marmorvej 51, 2100 Copenhagen, Denmark 5: National TB Reference Laboratory, National Center of Pulmonology SNCO, MH, Armenia 6: WHO-EURO laboratory consultant, National Center for TB and Lung Diseases of Georgia

Background:

Drug susceptibility testing (DST) is a critical component of tuberculosis (TB) control by allowing clinicians to select effective treatment regimens, thereby minimising the selection and transmission of drug resistance (DR). With estimated new and previously treated TB cases with DR-TB, which is 19% and 48% respectively and smaller number of lab-confirmed cases in 2021, DR-TB remains a public health concern in Armenia. To ensure the quality and reliability of DST results in Armenia, we conducted a comprehensive quality assurance (QA) intervention plan.

Methods:

59 presumed MDR *Mycobacterium tuberculosis* complex (Mtbc) strains isolated in Armenia in 2022 were tested by routine genotypic and phenotypic DST (g/pDST) at the Armenian reference laboratory. Results were complemented with pDST and whole-genome sequencing (WGS) based resistance prediction at the Supranational Reference Laboratory in Borstel, Germany.

Results:

High concordance between the pDST results of the two NRLs was observed for isoniazid (H), rifampicin (R), moxifloxacin (Mfx), bedaquiline (BDQ), linezolid (Lzd), clofazimine (CFZ), Ethambutol (E) and delamanid (Dlm). All borderline *rpoB* mutations were detected by Xpert. The WGS result were crucial to interpret the pDST results for E, pyrazinamide (Z), BDQ and CFZ.

Conclusion:

Results of WGS allowed to assess the accuracy of pDST to E, Z, BDQ and CFZ. Quality-assured DST results and a strategy to implement all recommended genotypic methods in countries with growing MDR rates should be prioritized. Overall, the need for additional, comprehensive and standardized, QA assessments was demonstrated to complement the annual WHO external quality assessment.

P59

Is stool a good specimen for diagnosing pulmonary tuberculosis in a high resource setting?

D B Folkvardsen [1] V N Dahl [1 2] T Lillebaek [1 3]
1: International Reference Laboratory of Mycobacteriology, Statens Serum Institut, Copenhagen, Denmark 2: Department of Infectious Diseases, Aarhus University Hospital, Aarhus, Denmark 3: Global Health Section, Department of Public Health, University of Copenhagen, Denmark

Sputum sampling for *M. tuberculosis* (Mtb) may be challenging in clinical practice. Because sputum is swallowed, acid-fast bacteria may potentially be detected in stool, providing an easily accessible specimen, especially in low-resource settings.

To investigate stool as a useful alternative to respiratory specimens in a resource rich tuberculosis (TB) low-burden setting, we examined one year of diagnostic specimens sent for mycobacteria investigations in Denmark. Of 14,966 specimens sent to the International Reference Laboratory of Mycobacteriology in Denmark during 2022, 306 were stool specimens received from 147 unique patients. In total, four of these from two different patients were Mtb culture positive. Both patients also had Mtb culture positive pulmonary specimens. In addition, eight of 136 (5.9%) stool culture negative patients had a culture positive respiratory specimen, two with non-tuberculous mycobacteria and six with Mtb (4.4%), respectively. The other 128 stool negative patients had no positive specimens. Of these, 47 only had stool sent for examination. Of 182 Mtb-PCR tested stool specimens, only three (1.6%) were positive, all also culture confirmed.

In conclusion, we found no added value of examining stool specimen from patients with pulmonary TB (PTB). All patients identified through stool also had positive respiratory specimens. In addition, some Mtb positive patients identified through respiratory specimens had positive stool samples. Thus, we discourage screening for PTB through stool specimens or examining stool specimens only, as this potentially leads to missed diagnoses of PTB, and advocate that the use of stool sampling for PTB is highly questionable in our setting.

P60

Shikimic acid amides as promising antitubercular agents – synthesis and *in vitro* studies

V Valcheva [1] I Slavcheev [2] S Dimitrov [1] T Pencheva [3] G Dobrikov [2]
1: The Stephan Angeloff Institute of microbiology, Bulgarian Academy of Sciences 2: Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences 3: Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences

Despite the significant progress in the development of new drugs against tuberculosis, many therapies and preventive measures do not lead to the expected favorable health outcomes for various reasons. Therefore, the discovery and/or synthesis of new potent and less toxic anti-TB compounds is very important. Here we present the synthesis, *in vitro* and *in silico* studies of 12 shikimate kinase derivatives that are vital for the metabolism of plants, bacteria and fungi but are completely absent in mammals and humans. Many of the compounds showed very good antimycobacterial and antifungal activities with MIC values ranging from 0.07–0.32 μM, which were comparable to those of isoniazid and amphotericin B. Promising docking scores were

obtained for all compounds in different protein targets (PDB ID 1NYT (*E. coli*), 1XAJ (*S. aureus*), 4BQS (*M.tuberculosis*)) confirming the results from *in vitro* experiments. The newly synthesized shikimic analogues showed significant antibacterial activity comparable to reference antibiotics and could be suggested for further pharmacological studies. Acknowledgements: We gratefully acknowledge support from Bulgarian Science Foundation (Grant KP-06-H39/7).

P61

In vitro susceptibility testing of GSK656 against *Mycobacterium tuberculosis complex* isolates to establish the epidemiological cut-off values and MIC distribution

A Ghodousi [2] I Iannucci [1] F Saluzzo [1 2] A Yepes [3] D M Cirillo [1 2]
1: Universita Vita-Salute San Raffaele, Milan, Italy 2: IRCCS San Raffaele Scientific Institute, Milan, Italy 3: Global Health Medicines R&D, GlaxoSmithKline, Tres Cantos, Madrid 28760. Spain

GSK656 is a novel Benzoxaborole targeting *Mycobacterium tuberculosis* complex (MTBC) leucyl-tRNA synthetase. The proposed use of GSK3036656 in Phase 2 combination regimen studies calls for the establishment of a robust phenotypic drug susceptibility testing (DST) method and a properly set breakpoint. A first step in this direction is the establishment of an *in vitro* standardized test based on the EUCAST protocol and the identification of the MIC distribution for the reference strain H37Rv ATCC 27294 and phylogenetically diverse clinical isolates.

To reach this objective, we tested H37Rv ATCC 27294 (31 independent replicates) and a panel of 25 phylogenetically diverse clinical isolates, showing different phenotypic resistant pattern to 1st and 2nd line drugs, using serial 2-fold dilutions from 0,004 to 0,5 mg/mL. Isoniazid was used as control drug against the H37Rv strain (serial 2-fold drug dilutions 0,008 to 1 mg/mL).

We observed a similar distribution of GSK656 MICs for H37Rv and the phylogenetically diverse clinical isolates, without no verifiable associated lineage effect. The MIC mode and the provisory ECOFF were identified respectively at 0.06 and 0.125 µg/mL (Fig 1).

Moreover, the two-laboratory derived low/high level resistant isolates showed an MIC of 0,12 and 2 mg/ml as expected.

In conclusion, based on the identified MIC distribution we propose a provisory critical concentration of 0.125 µg/mL for GSK656.

P62

The Niger rifampicin-resistant tuberculosis treatment approach is safer than the WHO bedaquiline/linezolid-containing 9-month regimen

M B Souleymane [1] T Decroo [2] I M Lawan [1] A Soumana [3] R H Moussa [4] N O Gutiérrez [5] S Mamadou [6] B C de Jong [7] L Rigouts [7] A Van Deun [8] A Piubello [5]
1: Damien Foundation, Niamey, Niger 2: Institute of Tropical Medicine, TB-HIV Unit, Antwerp, Belgium 3: National Tuberculosis Programme, Coordination, Niamey, Niger 4: Centre Hospitalier Regional de Tahoua, CAT, Tahoua, Niger 5: Damien Foundation, Brussels, Belgium 6: Université Abdou Moumouni de Niamey, Faculté des Science de la Santé, Niamey, Niger 7: Institute of Tropical Medicine, Unit of Mycobacteriology, Antwerp, Belgium 8: Independent Consultance, Leuven, Belgium

Bedaquiline (BDQ)/linezolid (LZD) all-oral short treatment regimens (AO+LZD-STR) are recommended for rifampicin-resistant tuberculosis (RR-TB) by WHO. The Niger RR-TB treatment strategy uses a second-line injectable drug (SLID)-STR, where the SLID is replaced by LZD when any grade of ototoxicity is identified on monthly audiometry. We compared both approaches through the SHOORT (SHOrt ORal Treatment) randomised clinical trial. Here we report the interim analysis for the occurrence of grade 3-4 adverse events (AE). Directly observed treatment and active drug safety and monitoring were applied throughout treatment duration. Monitoring procedures are adapted to fit the regimens (e.g. close monitoring of haemoglobin for patients taking LZD; monthly audiometry when a SLID is used). Between April 2021 and July 2022, 91 patients were enrolled. Baseline characteristics were similar for both arms. After excluding grade 3-4 AE that were attributed to a non-TB drug (n=1), 18 patients had at least one grade 3-4 AE: 5 of 46 (10.9%) treated with the Niger treatment strategy, and 13 of 45 (28.9%) treated with the all-oral STR (p=0.03). These 18 patients had 21 grade 3-4 AE (anaemia: 10, neurotoxicity: 6, hepatitis: 5, ototoxicity: 0). Ototoxicity and nephrotoxicity appeared during SLID-STR but none evolved to grade 3. Anaemia and neurotoxicity, including grade 3-4, were more frequent during treatment with the AO+LZD-STR. In conclusion, our interim data show that the Niger approach is associated with less grade 3-4 AE than the BDQ/LZD-containing all-oral STR. Findings need to be confirmed in the final analysis, expected by the end of 2023.

P63

Exploring the impact of mutations in *Rv0678* gene on bedaquiline resistance in *Mycobacterium tuberculosis*: insights from computational biostructural proteomics

J Snobre [1 2 3] L Rigouts [1] B C de Jong [1] O Tzfadia [1]
1: Mycobacteriology Unit, Biomedical Sciences, Institute of Tropical Medicine (ITM), Antwerp, Belgium 2: Doctoral School of Life Sciences & Medicine, Vrije Universiteit Brussel , Brussels, Belgium 3: Internal Medicine Department, UZ Brussel, Brussels, Belgium

Drug-resistant tuberculosis is a significant global health concern. Bedaquiline (BDQ) is a relatively new drug that targets *Mycobacterium tuberculosis* by disrupting its respiratory chain. Resistance to BDQ has been linked to mutations in the *Rv0678* gene, which regulates the efflux pump MmpS5-MmpL5. Previous attempts to identify *Rv0678* genomic hotspots with phenotypic resistance to

BDQ have not been successful, possibly due to the limitation of only considering linear sequence (1D) rather than potential mutations clusters on the protein tertiary structure (3D) as well as not taking into account alternative reading frames.

In this study, we used computational tools such as PDBpisa, Alpha Fold2 and SurfMap on a dataset of 224 isolates including in vitro selected and clinical isolates presenting a *Rv0678* mutation to study the impact of SNPs and frameshift mutations on the protein structure and final impact on BDQ MIC.

Results suggest the existence of mutations clustering in the Rv0678 tertiary structure, corresponding to the DNA-binding domain. Analysis of frameshift mutations in clinical isolates shows that they are primarily located in the initial 200 nucleotides of the protein. We also found that despite significant changes to the primary amino-acid sequence, some frameshifted proteins encoded by alternative reading frames have similar structures to the wild-type protein. Finally, different mutations clustering patterns in vitro selected and clinical isolates suggest that in vivo factors may have an impact on mutants' selection. Overall, our findings help bridge the genotypic-phenotypic gap in BDQ resistance and advance toward the development of a diagnostic molecular test.

P64

Prolonged heating should be avoided during the preparation of Delamanid containing Middlebrook 7H11 medium

P Rupasinghe [2] R Raeneers [2] J Vereecken [2] S Sengstake [2] L Rigouts [1 2] B C de Jong [2]
1: University of Antwerp, Wilrijk, Belgium 2: Unit of Mycobacteriology, Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium

Prompted by the detection of false resistance, we assessed the impact of four in-vitro conditions on the minimal inhibitory concentration (MIC) of delamanid against *Mycobacterium tuberculosis* complex (MTBc) on Middlebrook 7H11 medium. We tested the H37Rv reference strain with two different inoculum concentrations (10^{-1} and 10^{-2} of McF 1.0), using polystyrene vs polypropylene labware and, using delamanid-containing media protected from light and exposed to natural light minimum for six hours. Additionally, the impact of duration to heat exposure (51°C,<40 mins vs 4-5 hours) during medium preparation before aliquoting in the tubes was tested with an extended panel of strains including H37Rv.

There was no significant impact of the type of labware used or light exposure of the drug-containing medium on the delamanid MICs for the H37Rv strain. Higher inoculum concentration (10^{-1} of McF 1.0) didn't affect the MICs of the H37Rv strain, yet reduced the turnaround time of the results by seven days and the occurrence of invalid results due to lack of growth on the growth controls. A systematic increase in the growth of MTBc on the media exposed to 51°C for 4-5 hours was observed compared to the media with ≤40 mins exposure, leading to false-resistant delamanid results for the H37Rv strain. Our results suggest that the length of delamanid-containing media in the water bath at 51°C should be as short as possible in order to avoid false-resistant results. Thus strict adherence to the media preparation standard operating procedures is crucial to avoid unreliable results.

P65

Screening for non-fixed/mixed single nucleotide polymorphisms (SNPs) in serial *Mycobacterium tuberculosis* isolates during treatment for MDR-TB, the emergence of *sugI* mutations in patients receiving d-cycloserine.

R M Anthony [1] M Molemans [2] J Alffenaar [3 4 5] O Akkerman [6] M GG Sturkenboom [10] A Mulder [1] R de Zwaan [1] F CM van Leth [7] S Ghimire [10] N Yatskevich [8] A Skrahina [8] V Crudu [9] N Ciobanu [9] N Turcan [9]

1: National Tuberculosis Reference Laboratory, Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), 3721BA Bilthoven, the Netherlands 2: AIGHD Amsterdam Public Health Research Institute, Amsterdam, Netherlands 3: Sydney Institute for Infectious Diseases, The University of Sydney, Sydney, NSW, Australia. 4: School of Pharmacy, The University of Sydney Faculty of Medicine and Health, Sydney, NSW, Australia. 5: Westmead Hospital, Sydney, NSW, Australia. 6: Department of Pulmonary Diseases and Tuberculosis, University Medical Center Groningen, University of Groningen, Haren, The Netherlands, Tuberculosis Center Beatrixoord, University Medical Center Groningen, University of Groningen, Haren, The Netherlands 7: Department of Health Sciences, Vrije Universiteit Amsterdam 8: TB-NET 9: Phthisiopneumology Institute, Chi?inau, Republic of Moldova 10: Department of Clinical Pharmacy and Pharmacology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

This study aimed to investigate the adaptation of multi-drug resistant *Mycobacterium tuberculosis* (MDR-TB) during treatment.

Multiple *M. tuberculosis* DNA samples were collected from 54 patients at diagnosis and between 7 days and two months of MDR-TB treatment, shortly after the publication of the 2019 WHO consolidated guidelines on drug-resistant tuberculosis treatment in two sites.

Whole Genome Sequencing (WGS) data from 120 isolates was obtained and screened for unfixed single nucleotide polymorphisms (SNPs / mixed loci). Confidently mixed SNPs were identified in samples from multiple patients in only five genes (*gyrA*, *pncA*, Rv1129c [*prpR*], Rv1148c, and *sugI*), all other genes with confidently mixed SNPs were identified in isolates from only a single patient. Three different mixed SNPs were identified in the *sugI* gene from follow up isolates from three different patients (*sugI* - P7A, P7T, and Q6stop).

Mutations in *sugI* have been previously reported in spontaneous in vitro d-cycloserine resistant mutants. Alterations in *sugI* may indicate a sub-optimal treatment regimen containing d-cycloserine and potentially be of clinical significance with respect to adaptation to d-cycloserine. The majority of patients (52 of 54 patients) received d-cycloserine as part of their initial treatment. D-cycloserine was used within a new bedaquiline containing regimen at one site but as mainly within of regimens not containing bedaquiline at the second site.

Monitoring the accumulation of low frequency escape mutants has the potential to identify weaknesses in regimens and identify key drugs at risk of resistance selection.

P66

11-year trend in antibiotic consumption in Albania and the implications for the future

I Hoxha [1] A Malaj [1]
1: University of Medicine Tirana

There are growing concerns with rising antimicrobial resistance (AMR) across countries. These concerns are enhanced by increasing and inappropriate utilization of 'Watch' antibiotics with their greater resistance potential, and further exacerbated by increasing use of antibiotics to treat patients with COVID-19 despite little evidence of bacterial infections. Currently, little is known about antibiotic utilization patterns in Albania in recent years, including the pandemic years. In addition, the influence of an ageing population as well as increasing GDP and greater healthcare governance. Consequently, total utilization patterns in the country were tracked from 2011 to 2021 alongside key indicators. Key indicators included total utilization as well as changes in the use of 'Watch' antibiotics. Antibiotic consumption fell from 27.4 DIDs (Defined Daily Doses per One Thousand 1000 Inhabitants per day) in 2011 to 18.8 DIDs in 2019, which was assisted by an ageing population and improved infrastructures. However, there was an appreciable increase in the use of 'Watch' antibiotics during the study period. Their utilization rose from 10% of the total utilization among the top 10 most utilized antibiotics (DID basis) in 2011 to 70% by 2019. Antibiotic utilization subsequently rose after the pandemic to 25.1DIDs in 2021, reversing previous downward trends. Alongside this, there was increasing use of 'Watch' antibiotics, which accounted for 82% (DID basis) of the top 10 antibiotics in 2021. In conclusion, educational activities and antimicrobial stewardship programs are urgently needed in Albania to reduce inappropriate utilization including 'Watch' antibiotics and hence AMR.

P67

Genotypic diversity of strains of *Mycobacterium tuberculosis* isolated from TB patients from high burden MDR-TB country

N Ciobanu [1 2] A Codreanu [1] N Turcan [1] E Noroc [1 2] D Chesov [2] D Rusu [1 2] T Cohen [3] V Crudu [1 2]
1: Institute of Phthisiopneumology, Chisinau, Moldova 2: State Medical and Pharmaceutical University, Chi?inau, Moldova 3: Department of Epidemiology of Microbial Diseases, Yale School of Public Health, New Haven, Connecticut, USA

Background. The prevalence of multidrug and extensively resistant tuberculosis (M&XDRTB) is a serious problem, having major public health and economic consequences in the R. Moldova. Drug resistance, particularly M&XDRTB are associated with genotype of Mycobacterium tuberculosis.

The aim of this study was the prospective analysis of the sequencing results of the strains and determine the genetic lineages diversities of Mycobacterium tuberculosis related to drug sensitive and drug resistance strains among TB patients on the high burden MDRTB country.

Methods. We analyzed the results of sequencing of 4176 M.tuberculosis strains, isolated during 2010 -2019 years from new and previously treated TB patients from different territories of Moldova. From these 52.5% (n=2191) were susceptible strains and 47.5% (n=1985) were resistant strains.

Results. The results ofr the sequencing from sensitive strains: the predominance lineage was Lineage 2.2.1 – 31.4% (n-687), Lineage 4.8 –18.8%, (n=412), Lineage 4.2.1 – 14.6% (n=320) and Lineage 4.1.2 – 11.4% (n=249). Among the resistance strains were found two predominance lineages (95%): Lineage 4.2.1 – 48.3%, (n-958) and Lineage 2.2. 1– 45.8%, (n=909).

Conclusion. Lineage 4.2.1 (Ural) and Lineage 2.2.1 (Beijing) of M.tuberculosis are predominating among the resistance strains and significantly are associated with MDR&XDRTB.

P68

Elucidating drug tolerance and resistance mechanisms of *M. tuberculosis* using evolutionary medicine principles

A Hintz-Rüter [1] T Walz [1] L Sonnenkalb [1] S Niemann [1]
1: Research Center Borstel

Drug-resistant tuberculosis continues to be a public health crisis, challenging the existing treatment regimens with about half a million new cases, annually. The implementation of new regimens is not outpacing the rate of resistance development. To understand resistance evolution, we established several *in vitro* models for *Mycobacterium tuberculosis* complex (Mtbc) strains employing principles of evolutionary medicine. Evolutionary medicine applies natural selection concepts to understand and improve medical research. Exerting Next Generation Sequencing technology and *in vitro* assays, we gathered insights into resistance and resilience mechanisms, which may be used to advance evolutionary-informed treatment strategies. Further, we aim to understand the impact of the genetic background of clinical Mtbc strains on drug resilience development.

First results showed that resistance in Mtbc strains is selected at levels, far below the MIC of the wild-type. This indicated a large mutant selection window, which also varies for different resistance associated variants even within the same gene. Transcriptional profiling of selected mutant clones allowed a further elucidation of resistance mechanisms. To analyze the role of drug resilience in resistance development of Mtbc strains, we established *in vitro* models analyzing clinical Mtbc strains in response to key TB drugs. Ultimately, we hope to find intervention opportunities to curtail this resilience and resistance evolution.

By advancing our knowledge of resistance evolution, these models have the potential to expand treatment strategies utilizing evolutionary-informed approaches.

P69

Use of *M. tuberculosis* genome sequencing to determine relapse and reinfection in a phase 2 prevention of recurrent tuberculosis vaccine trial

A M Cabibbe [1] V Batignani [1] F Di Marco [1] D M Cirillo [1] The POR TB Consortium [2]
1: San Raffaele Scientific Institute 2: Statens Serum Institut

Whole genome sequencing (WGS) of *M. tuberculosis* (MTB) can distinguish relapse (same strain) from reinfections (different strains) in clinical studies, by comparing single-nucleotide polymorphism (SNP) distances between first and subsequent tuberculosis (TB) episodes. Here we assess strain identity by WGS for TB recurrence (relapses/reinfections) within a phase 2b prevention of recurrent (POR) TB vaccine trial and describe the phylogenetic features of the MTB study collection.

831 participants with active TB were enrolled from 6 sites in South Africa (SA) and Tanzania, randomized to receive H56:IC31 vaccine or placebo after successful treatment, and monitored for 12 months for recurrence. Sputum samples were provided for culture and Illumina sequencing at baseline and when TB symptoms occurred.

MTB genotyping data available for 631 (82%) baseline samples plus 30 recurrences showed significant associations (P ≤0.05) between MTB lineage (L) and geographic location: L3 in Tanzania, L4 and L2 at sites in Gauteng and Western Cape (SA), respectively. Recent transmission index was 14.4%. Recurrence was due to relapse in 17/30 (57%) events with average 0.46 SNP acquisition rate per 1 person-year, compared to 13/30 events (43%) suggestive of reinfection (average 678 SNP distance). Drug resistance shifting was not observed in relapses. L3 showed 8.2% relapse rate (P >0.05).

This trial is among the first to perform systematic WGS-based assessment of recurrence following a first TB episode in Sub-Saharan Africa and showed diverse MTB populations and modest transmission rates at trial sites, and higher relapse than reinfection within 1 year follow up.

P70

Whole genome sequencing from clinical primary *Mycobacterium tuberculosis* liquid cultures: pushing the boundaries

A Dippenaar [1] E Costa Conceição [2] F Wells [2] A Paulse [2] T H Heupink [1] V Rennie [1] M De Diego Fuertes [1] R M Warren [2] A Van Rie [1]
1: University of Antwerp 2: Stellenbosch University

Whole genome sequencing (WGS) for personalized treatment should be accurate and have a short turnaround time. Until WGS directly from sputum samples is feasible, clinical primary liquid cultures (CPLCs) are an attractive alternative. *Mycobacterium tuberculosis* CPLCs often produce low-quality DNA due to contaminants and low biomass. We submitted 158 CTAB-extracted DNA

samples from CPLCs for WGS at a commercial service provider. Of the 158 samples, 52 (33%) passed quality control (QC) with a DNA Integrity Number (DIN) ≥7, 83 (53%) had a DIN of 6-6.9 ('on hold') and 23 (15%) a DIN <6 ('failed)'. Because DNA replacement would require a subculture and create a delay of 3-8 weeks, we reviewed the fragment size distribution, peak height, and graph shape of electropherograms of 106 samples with DINs <7. We decided to process 104 of 106 (98%) samples with DIN<7. Of the 156 samples processed by library prep, 137 passed library QC, 16 had low library concentrations, and three completely failed library QC. WGS was performed on all except the three samples that completely failed. Using the MAGMA pipeline only three of 153 (2%) analyses failed (coverage depth <15×). The other 150 (98%) samples had a median depth of coverage of 1002× and a median of 91% mapped reads. MAGMA generated an actionable drug-resistance profile for all 150 samples that passed WGS-QC. Our results demonstrate that the standard criteria for DNA and library QC may be too stringent as actionable genomic drug resistance profiles from almost all (95%) CPLC samples were obtained.

P71

Performance of targeted and whole genome sequencing for routine genotypic drug resistance profiling of *Mycobacterium tuberculosis*

M De Diego Fuertes [1] A Dippenaar [1] E Costa Conceição [2] F Wells [2] A Paulse [2] T Tu [1] V Rennie [1] R M Warren [2] A Van Rie [1]
1: University of Antwerp 2: Stellenbosch University

Interest in Next-Generation sequencing (NGS) to determine the comprehensive drug resistance profile of *Mycobacterium tuberculosis* is increasing, but it remains unclear which application is most suited for high-burden countries. We compared the performance of whole genome sequencing (WGS) and targeted deep sequencing (tNGS) in 67 consecutive patients diagnosed with rifampicin-resistant tuberculosis (RR-TB) by Xpert MTB/RIF Ultra. Patients were predominantly HIV positive (72%) and 40% had prior TB treatment. Deeplexâ Myc-TB (tNGS) was performed on DNA extracted from NALC-NaOH sputum sediments. The MAGMA pipeline was used to analyse WGS for DNA extracted from clinical primary liquid cultures (CPLCs) of the same sediment. For the 67 patients, WGS/MAGMA was successful in 52 (78%) and Deeplex in 46 (69%). For 45 patients where both methods were successful, drug resistance profiles were identical in 35 (78%) but differed for one drug in five (11%) cases, and for >1 drug in another five (11%). Discordances in resistance calling were due to a difference in *ethA* variant classification (n=1), presence of variants outside of Deeplex targets: upstream of *inhA* (n=3) and *embA* (n=1), large genomic deletions in *katG* (n=1) or *pncA* (n=1), and minor variants (2-4% allele frequency) detected by only one method (n=5): three detected by Deeplex but missed by WGS/MAGMA, and two identified by MAGMA but missed by Deeplex. Results of this small study suggest an increased yield for culture-based WGS, small differences in resistance profile between sputum-based tNGS and CPLC-based WGS, low prevalence of hetero-resistance, and no evidence of clinically relevant culture bias.

P72

Advancing TB Diagnosis in Sub-Saharan Africa: A Roadmap for Next-Generation Sequencing Implementation

L de Araujo [1] C Utpatel [1] V Dreyer [1] A Cabibbe [2] T Niemann [1] V Mohr [1] C Gerlach [1] T Kohl [1] D Sinbandze [5] M Ziyane [5] T Fernando [6] C Abujate [6] L Mhuulu [7] A Diergaardt [7] A Kay [5] S Dlamini [3] T Dlamini [3] G Günther [8] M Claassens [7] E Nepolo [7] N Ismael [6] S Viegas [6] D Cirillo [2] S Niemann [1]

1: Molecular Mycobacteriology, Research Center Borstel - Leibniz Lung Center, Borstel, 23845, Germany 2: Emerging Bacterial Pathogens Unit, IRCCS San Raffaele Scientific Institute, Milan, 20132, Italy 3: National TB Reference Laboratory, Eswatini Health Laboratory Services, Eswatini. 4: – Department of Global Health, Amsterdam UMC, University of Amsterdam, The Netherlands 5: Baylor College of Medicine Children's Foundation, Eswatini 6: Instituto Nacional de Saúde, Marracuene, Mozambique. 7: Department of Human, Biological & Translational Medical Sciences, School of Medicine, University of Namibia, Windhoek, Namibia 8: Department of Pulmonology and Allergology, Inselspital, Bern University Hospital, University of Bern, Bern,3010, Switzerland

Targeted next-generation sequencing (tNGS) from clinical specimens has the potential to become a comprehensive tool for a broader, routine drug-resistance prediction of *Mycobacterium tuberculosis* complex (MTBC) strains, the causative agent of tuberculosis (TB). However, TB mainly affects low- and middle-income countries (LMIC) , which have specific needs and challenges for implementing new technologies.

Within the framework of the German Global Health Protection Programme, we share our experience on using a model for programmatic implementation of tNGS in three LMIC: Eswatini, Namibia and Mozambique. A roadmap of the implementation process will be herein described, this includes the budget/costs, strategic plan, list of equipment and other materials, trainings, and a SWOT analysis.

After successful capacity building of wet and dry lab infrastructure, the local team received hands-on on-site and on-line trainings on all needed steps from DNA extraction to sequencing on the iSeq100 sequencer. Multiplex PCR was based on a commercial test, the Deeplex®-Myc-TB, which was selected because of its capacity to deep sequence 18 MTBC gene targets that are associated with 13 first- and second-line drugs and includes mycobacterial species identification. Upon completion of the practical trainings, strains from clinical culture samples from TB patients were sequenced in pilot runs.

tNGS was successfully implemented in Eswatini, Namibia and Mozambique and is capable of providing additional clinically-relevant information, such as genotypic resistance profiles, lineage, and mixed infection information. Our next step is to proceed with the programmatic adoption of tNGS data in clinical practice and national guidelines.

P73

SARS-CoV-2 lineages and Variants Of Concern in patients from a university hospital in Tirana, Albania, January-February 2021

A Spitaleri [1] F Saluzzo [1] S Battaglia [1] V Poletti de Chaurand [1 2] A M Cabibbe [1] S Bino [4] S Bala [5] H Hafizi [5] P Kapisyzi [5] L Suraj [3] A Vasili [4] D M Cirillo [1] S Tafaj [3]
1: Emerging Bacterial Pathogens Unit, Division of Immunology, Transplantation and Infectious Diseases, IRCCS San Raffaele Scientific Institute, Milan, Italy. 2: Dipartimento di Medicina e chirurgia, Università degli studi di Milano Bicocca, Milan, Italy. 3: Microbiology Department National TB Reference Laboratory, University Hospital "Shefqet Ndroqi", Tirana, Albania. 4: Control of Infectious Diseases Department, Institute of Public Health,Tirana, Albania. 5: Pneumology Service, University Hospital "Shefqet Ndroqi", Tirana, Albania.

The emergence of SARS-CoV-2 Variants Of Concern (VOC) has boosted the attention to the molecular characterization of the virus. Sequencing and data sharing proved essential for tracing the origin and spreading of the virus mutations over time.

From the first week of January to the first week of March 2021, Albania reported 53087 confirmed cases and 728 deaths due to COVID-19. For this study we randomly selected 122 nasopharyngeal specimens (NPS) among the patients SARS-CoV-2 positive hospitalized at University Hospital Shefqet Ndroqi (UHSN).

The aim of this study was to analyze the SARS-CoV-2 viral genomes from strains collected in Albania at the beginning of 2021, when the Alpha variant became a worldwide major public health concern. We compared performances and results of Illumina and Nanopore sequencing techniques using ARTIC protocol V3, performing analysis over different common investigative tasks. Finally, we provided an overview of the virus transmission in Albania.

Our results show a good agreement match between the two sequencing technologies, allowing to properly characterize the SARS-CoV-2 clades. In particular, both sequencing defined the *alpha* variant VOC N501Y, D614G and P681H on S-spike, have similar trend as function of Ct values and the tanglegram comparison have an entanglement of 0.17, demonstrating a perfect phylogenesis analysis match.

P74

A Virtual Molecular Biology Training Program for DNA Extraction and Whole Genome Sequencing of *Mycobacterium tuberculosis* Clinical Isolates

A Dippenaar [1] T Sylvester [2] C Ealand [3] N Ismail [2] F Wells [2] B Mann [2] J Loubser [2] A Van Rie [1] M Miller [2] N Rakotosamimanana [4] B D Kana [3] R M Warren [2]
1: University of Antwerp 2: Stellenbosch University 3: University of the Witwatersrand 4: Institut Pasteur de Madagascar

High tuberculosis burdens are found in settings where integrating *Mycobacterium tuberculosis* (*Mtb*) whole genome sequencing (WGS) into routine care, surveillance and research remains challenging. The COVID-19 pandemic prompted us to develop a virtual training program covering topics of good laboratory practice and safety, development of standard operating procedures (SOPs), DNA extraction, WGS, and bioinformatics analysis of *Mtb* WGS data. Trainees (n=21) from five Southern African institutes received curated theoretical content. Over a 12-week period, trainees studied an assigned weekly section, followed by a short assessment and targeted review during a weekly interactive virtual meeting with the instructors. For the practical wet-lab training component, a needs-assessment survey was performed and the online training was piloted with a few participants at two institutions. An SOP for DNA extraction, accompanied by video snippets, was developed and a lab(oratory)-in-a-box containing the necessary consumables and reagents was assembled. During the virtual training, a remote video system (nanny cam) was used so instructors could observe the procedures and provide real-time feedback to the trainees. This provided insight into the unique challenges faced when implementing new techniques in environments with varying levels of infrastructure and access to consumables. Training completion facilitated the submission of three scientific articles for peer review and sparked new collaborations on *Mtb* WGS. Collectively, our approach was successful in providing equitable access to high-quality training for individuals unable to attend in-person training and serves as a proof-of-concept of a virtual laboratory training model for resource-limited settings.

P75

Household and community-based transmission of *Mycobacterium tuberculosis* in Harare, Zimbabwe

M Chipinduro [1 2] V Dreyer [3 4] J Mutsvangwa [1] E Marambire [1] K Madziva [1] C Calderwood [1 5] L S Larsson [1] U Panzner [6] S Niemann [3 4] N Heinrich [6] K Kranzer [1 5 6]
1: Biomedical Research and Training Institute, Harare, Zimbabwe 2: Midlands State University, Gweru, Zimbabwe 3: Molecular and Experimental Mycobacteriology, Research Center Borstel, Borstel, Germany 4: German Center for Infection Research, Partner Site Hamburg-Lübeck-Borstel-Riems, Borstel, Germany 5: Department of Clinical Research, Faculty of Infectious & Tropical Diseases, London School of Hygiene & Tropical Medicine, London, United Kingdom 6: Department of Infectious Diseases and Tropical Medicine, Ludwig-Maximilian University, Munich, Germany

Tuberculosis is a public health concern. While household transmission of *Mycobacterium tuberculosis* complex (Mtbc) strains is the main rationale for tuberculosis screening among household contacts (HHC), Mtbc transmission also occurs outside households, in some

settings. We investigated community and household transmission among people with tuberculosis (index cases and their HHC) in Harare, Zimbabwe. Whole genome sequencing (WGS) was performed on Illumina NextSeq 500. MTBseq-based WGS data analysis was used for phylogenetic strain classification and cluster analysis based on a ≤5 single nucleotide polymorphisms (SNPs) difference. Two-hundred tuberculosis patients were investigated of which 63% (n=126) were men and the median age was 35.1 years (IQR: 26.8-42.7). Out of a total of 200 sequenced Mtbc strains, 92.5% (n=185) were from index cases and 7.5% (n=15) from HHC. The majority of Mtbc strains belonged to the Latin America/Mediterranean lineage (57%, n=114), followed by the Beijing lineage (9.5%, n=19). Overall, 17 clusters were identified with the largest cluster having six members. Of these, 11 (65%) clusters comprised index cases, only. Among 15 HHC strains, six (40%) were clustered with strains from their corresponding index cases and two (13%) with non-household index cases. The remaining seven (47%) sequences were ungrouped of which one became independently clustered with non-household index cases when clusters of ≤12 SNPs difference were considered. Our data indicate, that in Zimbabwe tuberculosis transmission occurs within households and in the community. HHC studies to validate early diagnostic tests must confirm that secondary tuberculosis cases are due to transmission within the household.

P76

Transmission and drug resistance surveillance by whole-genome sequencing in a population-based cohort in Southern Mexico

F J Martínez-Martínez [1] P Cano-Jiménez [1] V Furió [1 2] M Torres-Puente [1] G Delgado-Sánchez [3] L Ferreyra-Reyes [3] E Ferreira-Guerrero [3] M Bobabilla del Valle [4] A Ordaz [4] N Mongua-Rodríguez [3] N Tellez [3] S Canizales-Quintero [3] J Sifuentes-Osornio [4] L García-García [3] I Comas [1 5] M G López [1]
1: Instituto de Biomedicina de Valencia (IBV-CSIC) 2: Fundación para el Fomento de la Investigación Sanitaria y Biomédica de la Comunitat Valenciana (FISABIO-CSISP) 3: Instituto Nacional de Salud Pública (INSP) 4: Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (INCMNSZ) 5: Centro de Investigación Biomédica en Red en Epidemiología y Salud Pública (CIBERESP)

In contrast to global rates, tuberculosis in Mexico (25 cases/100,000) has increased since 2005. A population-based study was conducted in Southern Mexico during 1995-2010, including 1,018 sputum samples from 903 patients. Available data include: phenotypic drug-susceptibility testing (DST) using BACTEC for first-line antibiotics; molecular genotyping and clustering by MIRU-VNTR, and IS6110 RFLP/Spoligotyping.
Whole-genome sequencing (WGS) of this cohort using short-read Illumina technology is currently ongoing. A partial comprehensive genomic epidemiology study was conducted in 396 *Mycobacterium tuberculosis* complex clinical isolates. We performed: lineage typing by using phylogenetic SNPs; transmission investigation by SNP cut-off-based clustering method, and prediction of drug-susceptibility profiles by evaluating the mutations reported in the WHO Catalogue. Results from WGS were compared with molecular clustering and phenotypic DST. Analysis of the mycobacterial population structure revealed predominance of lineage 4 strains, particularly L4.1 (53%). Clustering was 47.5% (60 clusters) for 12 SNPs distance cut-off, ranging between 11.1-51.0% (0-15 SNPs); 47.2% for MIRU-VNTR (60 clusters, 20 of which were identical to WGS clusters); and 41.9% for RFLP/Spoligotyping (43 clusters). Mutations associated with resistance to isoniazid (INH) and rifampicin (RIF) were found in 12.6% and 5.5% of the isolates, respectively. Ten isolates (2.5%) were classified as MDR, most of them (8/10) in retreated cases.The specificity of WGS-based DST for INH and RIF was greater than 99%, while sensitivity

was between 81-86%. WGS could improve tuberculosis control in Mexico by providing an in-depth understanding of transmission.

P77

Epidemiology of infections caused by Tuberculous and Non-Tuberculous Mycobacteria in the province of Pavia: a 12-year comparative analysis

M Siciliano [1] V Monzillo [2] J Bagnarino [1] G Petazzoni [1] P Cambieri [1] F Baldanti [1]
1: U.O.C. Microbiologia e Virologia, Dipartimento Medicina Diagnostica, Fondazione IRCCS Policlinico San Matteo, Pavia, 27100, Italy 2: Dipartimento di Medicina Interna e Terapia Medica, Università degli Studi di Pavia, Pavia, 27100, Italy

Mycobacteria pulmonary diseases cause significant morbidity and mortality to human health. Although Tuberculosis (TB) remains a serious public health problem, the prevalence of lung diseases caused by Non-Tuberculous Mycobacteria (NTM) is increasing worldwide. Our study aims to illustrate the 12-year epidemiological changes of NTM and Mycobacterium tuberculosis complex (MTC) infection in the province of Pavia. The study was performed on 437 biological samples positives for MTC and 345 positives for NTMs, from 2011 to 2022. All samples were decontaminated with MycoTB™, according to current guidelines. Isolation and identification of strains were carried out with BACTEC MGIT™ 960 and GenoType AS/CM/NTM-DR/MTBC/GeneXpert, respectively. Patients were considered incident in the year in which they became culture positive. Our data showed a sharply increasing trend over the years of NTMs compared to MTC, which have declined, both for pulmonary and extra-pulmonary diseases. As regard pulmonary disease, MTC were isolated in 56% of cases, while NTM most frequent isolates were Mycobacterium avium complex (MAC) (n=152; 25,7%), M. abscessus complex (n=22; 3,7%) *M. xenopi* (n=23; 3,9%), *M. fortuitum* (n=8; 1,4%), *M. chelonae* (n=8; 1,4%) and *M. kansasii* (n=8; 1,4%). We also evaluated the distribution by age groups (0-18, 19-64, >65 years). MTC pulmonary infections were more frequent in 19–64-year-old, while those sustained by NTM were more widespread in over 65 year-patient. The increased trend for NTM could in part be explained by the rise of aged population with lung diseases such as BPCO and bronchiectasis, the employment of immunosuppressive drugs and the development of diagnostic techniques.

P78

WGS-based genetic diversity assessment of LAM genotype *M. tuberculosis* strains among the tuberculosis outbreaks in distant Latvian counties

D Sadovska [1 2] I Pole [1 3] I Ozere [2 3] A Viksna [2 3] I Norvaisa [3] R Ranka [1 2]
1: Latvian Biomedical Research and Study Centre, Riga, Latvia 2: Riga Stradins University, Riga, Latvia 3: Riga East Clinical University Hospital, Centre of Tuberculosis and Lung Diseases, Stopini region, Upeslejas, Latvia

Mycobacterium tuberculosis (Mtb) strains of the Latin American-Mediterranean (LAM) genotype family commonly cause cavitary tuberculosis (TB) and are associated with higher bacillary load. Although this genotype is widespread in Latvia, a little is known about its local genetic diversity. Herein, we delineated the transmission network of six local TB outbreaks caused by LAM Mtb strains that occurred in six distinct Latvian counties and assessed the genetic distances between Mtb isolates. WGS was performed on Mtb isolates obtained from epidemiological clusters of 13 (A), 12 (B), five (CDE), and three (F) patients diagnosed in 2004-2019; four patients had a recurrent episode. Isolates of four outbreaks (BCDE) belonged to the SIT254 spoligotype, while isolates of A and F outbreaks had SIT42 corresponding pattern. The maximum genetic distance between SIT254 Mtb isolates was 99 SNVs, while between SIT42 isolates it reached 112 SNVs. Within the clusters, all but one Mtb isolates exhibited pairwise distances of 0-11 SNVs. Notably, Mtb isolates of three geographically distinct epidemiological clusters formed a bigger outbreak with maximum distance of 16 SNVs between isolates. To conclude, results demonstrated genetic relatedness between all studied isolates belonging to two LAM family spoligotypes and highlighted a possible Mtb strain transmission between distant Latvian counties. This study was supported by ERDF grant No. 1.1.1.1/20/A/046.

P79

Relative competitive fitness of *Mycobacterium tuberculosis* outbreak strains isolated in Poland

K Strus [1] L Fiedorowicz [1] A Minias [1]
1: Institute of Medical Biology, Polish Academy of Sciences

Tuberculosis remains a highly prevalent disease and a significant contributor to worldwide mortality. The fight against TB requires surveillance of the population of the strains circulating worldwide. Currently, there is little knowledge of the characteristics of M. tuberculosis that cause it to be involved in outbreaks.This study aimed to answer if the relative fitness of M. tuberculosis strains might affect tuberculosis transmission. We identified six pairs of high- and low-transmission M. tuberculosis strains and transformed them with selective plasmids. Two bacterial strains, each representative of high- and low-transmission groups, were placed in one culture in a 1:1 ratio of colony-forming units. Culture samples were plated on selective media on days 0 and 7 to assess the ratio of colony-forming units. Next, the strains' relative competitive fitness (W) was calculated. The median relative fitness of high-transmission to low-transmission strains of M. tuberculosis was -0.23 (IQR -1.47-0.32). These results suggest that bacterial fitness

might not be associated with increased tuberculosis transmission. Our results require further confirmation with a larger sample of M. tuberculosis strains.

P80

Impact of *Mycobacterium tuberculosis* complex strain diversity on tuberculosis transmissions in a cosmopolitan low-incidence setting

N Ullrich [1] R Diel [2 3] F P Maurer [4] K Meywald-Walter [5] C Schwarzbach [5] M Kuhns [4] I Friesen [4] S Niemann [1 6] V Dreyer [1 6]

1: Molecular and Experimental Mycobacteriology, Research Center Borstel, Borstel, 23845, Germany. 2: Institute for Epidemiology, University Medical Hospital Schleswig-Holstein, Kiel, 24105, Germany. 3: LungClinic Grosshansdorf, Airway Research Center North (ARCN), German Center for Lung Research (DZL), Großhansdorf, 22927, Germany. 4: National and Supranational Reference Center for Mycobacteria, Research Center Borstel, Borstel, 23845, Germany. 5: Public Health Department Hamburg-Mitte, Hamburg, 20355, Germany. 6: German Center for Infection Research, Partner Site Hamburg-Lübeck-Borstel-Riems, Borstel, 23845, Germany

Understanding the factors driving tuberculosis (TB) transmission in a particular setting is essential to guide effective public health measures. Interestingly, only strains of some *Mycobacterium tuberculosis* complex (Mtbc) lineages occur globally (generalists), while others are only found in particular regions (specialists). This points towards differences in the Mtbc strain transmissibility, potentially influencing TB epidemiology in low-incidence settings by import of more transmissible strain types. To understand interactions between TB transmission, host and pathogen type in a low-incidence setting, we analysed notified TB cases in Hamburg, Germany, from 1997 – 2021 in relation to strain type and transmission data inferred from whole genome sequencing data of the 3062 Mtbc strains. Strains of Mtbc L1 to L6 and *M. bovis* were found in the strain collection, with a dominance of L4 strains (75%). Within L4, L4.1.2.1 (“Haarlem”) and L4.8 (“mainly T”) strains were most prevalent (32% and 20% of L4 strains, respectively) and also most frequently transmitted (30% and 18% of clustered L4 strains, respectively). Associations of ancestry and strain types, reflected in the transmission events were found at lineage and L4 sublineage level consistently over the entire 25-year period. For example, transmissions of L3 strains were mostly found between patients born in Eastern Africa, while L4.1.2.1 and L4.8 strains transmitted in patients with a wider range of origins. Our findings support the hypothesis that strains of generalist lineages can efficiently transmit in wider range of host genetics background. L4.8 strains appear to be an important widespread generalist lineage.

P81

Transmission of drug resistant tuberculosis in Mozambique

I Barilar [1 2] T Fernando [3] C Utpatel [1] C Abujate [3] C M Madeira [3] B Jose [4] C Mutaquiha [4] K Kranzer [5 6 7 10] T Niemann [1 2] N Ismael [3] L D Araujo [1] T Wirth [8 9] S Niemann [1 2 11] S Viegas [3]
1: Molecular and Experimental Mycobacteriology, Research Center Borstel, Borstel Germany 2: German Center for Infection Research, Partner Site Hamburg-Lübeck-Borstel-Riems, Borstel, Germany 3: Instituto Nacional de Saúde, Marracuene, Mozambique 4: National Tuberculosis Control Program, Directorate of Public Health, Ministry of Health, Maputo, Mozambique 5: Biomedical Research and Training Institute, Harare, Zimbabwe 6: Department of Clinical Research, Faculty of Infectious and Tropical Diseases, London 7: Division of Infectious Diseases and Tropical Medicine, University Hospital, LMU Munich, Munich, Germany 8: EPHE, PSL University, Paris, France 9: Institut de Systématique, Evolution, Biodiversité, ISYEB, Muséum national d'Histoire naturelle, CNRS, Sorbonne Université, EPHE, Université des Antilles, Paris, France. 10: School of Hygiene & Tropical Medicine, London, UK 11: Department of Human, Biological and Translational Medical Sciences, School of Medicine, University of Namibia, Windhoek, Namibia

With an estimated tuberculosis (TB) incidence of 368/100,000 and more than 4000 new rifampicin resistant (RR) or multidrug resistant (MDR) cases occurring every year, Mozambique is severely affected by the TB epidemic. However, detailed data on the drivers of the drug resistant (DR) TB epidemic in the country is not available.

To address this question, we performed whole genome sequencing (WGS) of 809 DR *Mycobacterium tuberculosis* complex (Mtbc) strains submitted to the National Tuberculosis Reference Laboratory in Maputo, Mozambique between 2015 and 2021. WGS data were used to perform phylogenetic lineage classification, DR profiling and transmission cluster analysis (12 SNP threshold).

628 strains were classified as MDR, out of which 21 % were pre-extensively drug resistant (XDR) due to a fluoroquinolone (FQ) resistance. 61 strains had a bedaquiline (BDQ) resistance with increasing prevalence over the study period. The cluster rate was high reaching 94% and 96% among pre-XDR and XDR Mtbc strains, respectively. Several large clusters with up to 72 isolates were detected. Particularly interesting were two outbreak clones. The first comprised 36 Ancestral 1 Beijing strains (2.2.2), which all had a FQ resistance and four an additional BDQ resistance. The second formed by 38 S-type strains (4.4.1.1), all with the RR *rpoB* I491F not detected by conventional molecular DR tests. Out of these, 19 were BDQ resistant, and 13 XDR.

In conclusion, transmission appears to be a main driver of DR-TB epidemic in Mozambique. Furthermore, public action has to be taken to control the emergence of pre-XDR/XDR-TB and "diagnostics escape" strains.

P82

Interrogation of an Multi Drug Resistant Tuberculosis outbreak using Whole Genome Sequencing

J Sims-Handcock [1] P Tshavhungwe [1] J Loubser [1] A Dippenaar [2] N Kriel [1] R Warren [1] E Streicher [1]
1: Stellenbosch University 2: University of Antwerp

The spread of drug-resistant strains which has been reported to be attributed to primary transmission threatens TB control and prevention programmes. Previous molecular epidemiological studies have reported that the dynamics of tuberculosis transmission varies geographically. After a reported increase in drug resistant TB cases in the West Coast region of the Western Cape Province, South Africa, the aim of this study was to identify transmission hotspots and possible outbreaks of drug-resistant tuberculosis within this region. Spoligotyping and Sanger sequencing of first line drug resistance conferring mutations of drug resistant strains in the region over 5 years (2008-2012) identified an MDR-TB outbreak of the X-family mainly located in the Northern parts of the region. Whole genome sequencing (WGS) were done on all available strains (n=177) to establish the phylogenetic relationships of this outbreak. Through WGS and sanger sequencing we found identical mutations conferring resistance to the 4 first-line drugs used in tuberculosis treatment in this lineage 4.1.1 cluster, including a rare katG315 double mutation. This is indicative of transmission of MDR-TB. Isolates belonging to this outbreak, but with different additional mutations conferring to resistance to second-line drugs were also identified, indicating that Pre-XDR-TB are primarily acquired on an existing MDR strain genotype. XDR-TB has not yet been seen, as this outbreak peaked before the introduction of new generation anti TB drugs. Monitoring and interrogation of drug resistant TB outbreaks plays an important part in our understanding of the drug resistant TB epidemic to ultimately eradicate TB disease worldwide.