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# **Scientific Program including Abstracts**

in the clinical outcome of the disease and to drug resistance. We genotyped 139 clinical isolates of *M. tuberculosis* obtained from patients of pulmonary tuberculosis and attending a referral center for patients with respiratory diseases in Delhi, India. The isolates were analyzed using nine Single Nucleotide Polymorphism (SNP) markers. The isolates were also subjected to spoligotyping and MIRU-VNTR typing; and the results correlated with their drug susceptibility profile.

The most predominant cluster was SNP cluster group (SCG) 3a observed in 56% (n=78) of the isolates. Majority (50%) of the isolates in SCG 3a were drug susceptible, while 9% (n=7) were multidrug resistant (MDR). The ancestral cluster SCG 1 was observed in 18% (n=25) of the isolates. Multidrug resistance was observed in 8% (n=2) of these isolates while 40% (n=10) of the isolates were drug susceptible. SCG 2 formed 6.2% (n=9) of the isolates and 44% (n=4) of these were MDR. Spoligotyping subdivided the strains into 45 shared types (n=125) and 14 orphan strains. The orphan strains were mostly associated with SCG 3a or SCG 1, reflecting the principal SCGs found in the Indian population. SCG 1 and SCG 2 spoligotypes were concordant with the East African Indian and Beijing families respectively. Central Asian (CAS) clade and its sublineages were predominantly associated with SCG 3a, however a few strains (n=3) were also observed to be SCG 3b. No consistent association was seen between the SCGs and other spoligotypes such as Harlem, T and X clades. Surprisingly, the 15 loci MIRU-VNTR typing revealed all isolates to be unique.

In conclusion, though our study revealed the preponderance of SCG 1 and 3a in the *M. tuberculosis* population circulating the region, the diversity of strains highlights the changes occurring within lineages and reemphasizes the importance of cluster investigations.

## P219

**STUDYING THE METABOLIC ACTIVITY OF MYCOBACTERIUM TUBERCULOSIS THROUGH A NEW RESAZURIN REDUCTION BASED ASSAY**  
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Lately, several approaches have been performed to study the biological aspects of the mycobacterial metabolism and elucidate some bacterial strategies to survive to host responses, but little is known about

mycobacterial adaptation to adverse environmental conditions. In this study we developed a simple and rapid method to evaluate the metabolic rate of *Mycobacterium tuberculosis* (*M. tuberculosis*) strains in liquid culture media, based on the resazurin reduction kinetics.

We tested four mycobacterial growth conditions: physiological (pH 6.7), acidic (pH 5.5 and pH 4.5) and anaerobic; the avirulent strain *M. tuberculosis* H37Ra was cultured until log phase, and 3 different bacterial concentrations ( $10^8$ ,  $10^6$  and  $10^5$  CFU/ml) were inoculated in triplicate, and incubated at 37°C with 5% CO<sub>2</sub> atmosphere. After inoculation, 20µl of resazurin 0.01% were added, and the indicated optical density was measured at several time-points (0h, 6h and 24h). This procedure was repeated three days after the inoculation, concluding a 5 day period of incubation. CFU counting was performed at the time of inoculation, and at the end of experience for each condition.

Globally, our results show an increasing resazurin reduction curve dependent on the normal growth curve of viable bacteria. However, after a three day incubation period the percentage of resazurin reduction kinetics was shown to be linear only for cultures inoculated with  $10^6$  CFU/ml. This pattern was also observed under acidic and anaerobic conditions. The method was found to be reproducible with low standard deviations. Thus, we selected the  $10^6$  CFU/ml as the best bacterial inoculum concentration for this assay. Afterwards, we analysed the growth curve of  $10^6$  CFU/ml culture for each conditions, and CFU counting as well. Although the H37Ra strain has the capability to grow in all conditions tested, which was confirmed by CFU counting, it was possible to distinguish the rate of resazurin reduction across the different environments. As expected, a smaller number of CFU/ml was obtained in acidic conditions when compared to anaerobic and aerobic conditions. Taking into account these results, we believe that the methodology here presented holds potential for mycobacterial metabolic studies concerning different strain physiology.

## P220

**IDENTIFICATION OF THE BEST MIRU-VNTR SET TECHNIQUE FOR MDR-TB AND XDR-TB SURVEILLANCE IN LISBON, PORTUGAL**  
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Multidrug and extensively drug resistance tuberculosis (MDR/XDR-TB) remains a serious threat to TB control worldwide. Portugal registers a high number of MDR-TB and XDR-TB cases particularly in Lisbon, the capital city. The majority of MDR- and XDR-TB cases that circulates in Lisbon belongs to either one of two groups of genetically close strains, known as Lisboa family and Q1 cluster. These strains are responsible for more than 80% of MDR-TB cases; 50% of which are also XDR-TB (2008). Given the high prevalence and considering the drug resistant profile of such strains, we aimed to investigate the most discriminatory technique for MDR- and XDR-TB surveillance.

For this purpose, 74 clinical *Mycobacterium tuberculosis* isolates collected in Lisbon Health Region were genotyped by 12, 15 and 24-loci *Mycobacterium interspersed repetitive units – variable number of tandem repeats* (MIRU-VNTR). Twenty-two isolates were susceptible and 54 were resistant to at least one drug (34 MDR-TB, 15 of which were XDR-TB). We verified that MIRU-VNTR analysis based on 12, 15 and 24 loci sets allowed the clustering of 22 (66,7%), 20 (60,6%) and 17 (51,5%) MDR-TB isolates; 13 (92,9%), 12 (85,7%) and 11 (78,6%) of these were also XDR-TB, respectively. It was also noticed that, as the number of analysed loci increased, the Lisboa3 and Q1 isolates that differ by one or two loci were gradually excluded from the clusters. In order to evaluate the clustering of MDR-TB and XDR-TB isolates, we analysed the discriminatory power of all MIRU-VNTR sets, applying the Simpson's Diversity Index. As expected, the 24 loci set analysis presented the highest discriminatory index (D) in comparison with both 12 and 15 loci sets ( $D_{MIRU\ 24} = 0,971$ ;  $D_{MIRU\ 15} = 0,964$ ;  $D_{MIRU\ 12} = 0,931$ ). The small difference observed between  $D_{MIRU\ 15}$  and  $D_{MIRU\ 24}$  leads to the conclusion that 15 loci MIRU-VNTR is a powerful discriminatory tool. Interestingly, although with a lower discriminatory power, the 12-loci MIRU-VNTR analysis was enough to cluster the XDR-TB isolates into two major clusters, Lisboa3 and Q1 cluster, corresponding to 92,9% of XDR-TB cases in Lisbon Health Region. Despite having a lower discriminatory power, we conclude that, in such settings, 15 loci MIRU-VNTR has a sufficient discriminatory power for MDR-TB and XDR-TB surveillance and that 12 loci MIRU-VNTR is a useful method to ascertain a common origin between drug resistant isolates that have undergone subsequent genetic diversification.

## P221

### DRUG RESISTANCE AND GENETIC DIVERSITY OF MYCOBACTERIUM TUBERCULOSIS IN LUANDA, ANGOLA: A MOLECULAR EPIDEMIOLOGICAL PERSPECTIVE

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Tuberculosis (TB) poses a serious public health problem in Angola. The latest estimates from the World Health Organization point to the occurrence of 89 000 new cases in 2013 and an incidence rate of 320 cases per 100 000 habitants in the same period. Furthermore, no surveillance data on drug resistance is available and nothing is known regarding the genetic diversity and population structure of circulating *Mycobacterium tuberculosis* (*M. tuberculosis*) strains. Its capital city, Luanda, harbours approximately 33.7% of the country's population and is responsible for one-third of the TB cases nationwide.

In the present study we have analysed 77 *M. tuberculosis* isolates recovered from sputum samples from the same number of patients. The patients involved in the study were clinically diagnosed with TB and positive for sputum smear acid fast bacilli in Hospital da Divina Providência, Luanda. All isolates were genotyped by Spoligotyping and 24-loci *Mycobacterium Interspersed Repetitive Unit – Variable Number of Tandem Repeats* (MIRU-VNTR). First-line drug susceptibility testing was performed by the standard BACTEC 960 MGIT procedure.

We have detected 29 different spoligotype profiles corresponding to 21 different Shared International Types (SIT) and 8 orphan profiles. SIT 42 (Latin American Mediterranean, LAM9) was the most prevalent SIT found (n=15, 19.5%) followed by SIT 53 (T1; n=11, 14.3%). Overall, the *M. tuberculosis* population structure in this sample was dominated by LAM (57.1%) and T (32.5%) strains. Twelve and 24-loci MIRU-VNTR analysis revealed that 47 (15 clusters) and 11 (4 clusters) isolates were clustered, respectively.

Drug susceptibility data showed that 17 (21.1%) of the 77 clinical isolates were resistant to one or more antibacterial drugs and from these, 3 (3.9%) were multidrug resistant (MDR). Drug resistant isolates were found across distinct clades and MIRU-VNTR clusters although the largest MIRU-VNTR cluster detected was comprised by 3/5 drug resistant isolates, including one MDR isolate



In conclusion, this study has demonstrated a high predominance of LAM strains circulating in Luanda and the presence of recent transmission events. The genetic diversity found suggests the presence of multiple and complex TB transmission chains masked by recent genetic diversification. The high rate of MDR-TB found in this sample has major public health implications and highlights the need of further studies specifically focused on MDR-TB transmission.

## P223

### CLUSTER IDENTIFICATION AND ANALYSIS OF CLINICAL MYCOBACTERIUM TUBERCULOSIS ISOLATES IN THE STATE OF HAWAII

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Hawaii's tuberculosis incidence rates are perennially among the highest in the United States. Immigration from the Western Pacific and Asia over the last decade continues to introduce a steady supply of new tuberculosis cases into the state, an average of 121 cases per year with an increase to over 138 cases this past year. This is in contrast to the declining number of cases in the US over the same period. As a result of this importation, distinguishing locally transmitted tuberculosis cases from cases brought into the state has been difficult when using traditional epidemiological methods.

In this study, we analyzed data covering 439 isolates collected by the Hawaii State Department of Health from 2004 to 2013 to identify potential transmission clusters. Genetic clusters were identified using spoligotyping and MIRU-VNTR fingerprinting methods.

Out of twenty genetic clusters consisting of three or more isolates, we identified six major fully fingerprinted clusters that included cases at least three years apart. Of these, two clusters were composed of isolates from the Beijing family and four clusters from the Manila family. One Beijing family cluster was composed of seven isolates and consisted primarily of US-born Micronesian males age 15-24, while the other had eleven isolates and consisted primarily of young to middle-aged people of Pacific Islander descent, but also included two North Korean-born women. Of the largest Manila family clusters, one had 23 isolates with six similar spoligotype patterns, while the other had 24 isolates with three similar spoligotype patterns. Both larger Manila family clusters were composed of persons born in the Philippines and covered a broad age range, with the 45-64 and 65+ age ranges being most common. Two more Manila family clusters were tentatively identified by their MIRU loci 1-12 patterns

that were shared with other Manila family clusters. One of these clusters had five cases, composed of four Micronesians and one US born case, while the other cluster had six cases including two cases born in the Marshall Islands, two Filipinos, one Micronesian and a US case.

Periodic evaluation of genotyping information has revealed possible transmission clusters and highlighted the diversity of tuberculosis cases in Hawaii. Linking genotypes to case data to determine potential epidemiologic links is essential as we move into whole genome sequencing of representatives of these clusters, currently in progress.