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MOLECULAR EPIDEMIOLOGY OF MYCOBACTERIUM TUBERCULOSIS COMPLEX IN THE CENTER OF TUNISIA (2008-2010 AND 2014-2017)

Ahmed Ben Hadj Hassine[1,2,3]*, Manel Marzouk[3], Jamal Saad[1], Jalel Boukadida[3] and Michel Drancourt[1]

[1]Institutes Hospital University Méditerranée Infection, Marseille, France
[2]Faculty of Pharmacy of Monastir, University of Monastir, Tunisia
[3]Laboratory of Microbiology and Immunology, UR12SP34, University Hospital Farhat Hatched Sousse, Tunisia

Corresponding author: benhadjhassine.ahmed@yahoo.fr

ABSTRACT

Tuberculosis remains one of the leading causes of morbidity and mortality in Arab Maghreb. In this large and diverse geographic region, the dynamic of tuberculosis is contrasted among the different countries. In Tunisia, tuberculosis mortality is decreasing, but its incidence has risen almost one and a half times between 2002 and 2016. To contribute to the knowledge of tuberculosis epidemiology in Center Tunisia, we performed a characterization of *Mycobacterium tuberculosis* complex mycobacteria isolated over 7 years to depict the dynamics of pulmonary tuberculosis in the region of Sousse and to establish molecular epidemiology. We investigated all the Ziehl-Neelsen-positive isolates made from respiratory tract specimens and related clinical specimens submitted for the routine diagnosis of pulmonary tuberculosis in one university and tertiary care center, Center Tunisia. After heat-inactivation, isolates were identified using *rpoB* gene sequencing first and quantitative real-time PCR (qRT-PCR) of the internal transcribed spacer (ITS) to confirm the identification of mycobacteria as members of the *M. tuberculosis* complex. Then, identification at the species level within the *M. tuberculosis* complex was ensured by PCR-sequencing of the Exact Tandem Repeat D. Finally, the M. tuberculosis Beijing family was identified by using qRT-PCR in the isolates identified as M. tuberculosis sensu strict, which were further genotyped using RD deletion region system to detect lineages. Among 298 *M. tuberculosis* complex isolates, 294 isolates were identified as *M. tuberculosis sensu stricto* and four isolates as *Mycobacterium bovis* BCG. The prevalence of *M. tuberculosis* in Beijing families steadily increased from 9.52% in 2014 up to 23.33% in 2016. *M. tuberculosis* was identified as the agent primarily involved in pulmonary tuberculosis and a correlation between the increasing prevalence of Beijing family isolates and increasing incidence of pulmonary tuberculosis was detected.

***Keywords*:** *Mycobacterium tuberculosis*, *Mycobacterium tuberculosis Beijing*, IS*6110* insertion sequence, Real-time PCR, ETRD, RD deletion

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1. INTRODUCTION

Tuberculosis remains one of the leading causes of morbidity and mortality in Arab Maghreb countries which is one of the endemic zones for tuberculosis (Ben Hadj Hassine et al. 2017; World Bank Group Data 2018). Arab Maghreb is a large and diverse geographic region where the dynamic of tuberculosis is contrasted among the different countries. For example, between 2002 and 2015, mortality increased from $3.6/10^5$ inhabitants to $11/10^5$ inhabitants in Libya. It remained stable at $8.1/10^5$ inhabitants in Algeria and decreased from $14/10^5$ inhabitants down to $9.6/10^5$ inhabitants in Morocco and from $4.8/10^5$ inhabitants down to $3.4/10^5$ inhabitants in Tunisia (Ahmed et al. 2017).

In Tunisia, in this context of decreasing mortality, the incidence of tuberculosis nevertheless rose from $24/10^5$ inhabitants in 2002 to almost one and a half times at $38/10^5$ inhabitants in 2016 (World Bank Group Data 2018). Tunisia is roughly comprised of three different regions with ecological and population specificities, i.e., North Tunisia, Center Tunisia, and South Tunisia. A dozen epidemiological studies of tuberculosis have been conducted over the last two decades in North Tunisia (Chevrel-Dellagi et al. 1993; Hermans et al. 1995; Karboul et al. 2008; Refai et al. 2015; Djemal et al. 2017), but only two studies have been conducted in South Tunisia (Smaoui et al. 2015; Siala et al 2017). As for Center Tunisia, only one study of human tuberculosis was conducted (Kahla et al. 2011) whereas three studies concerned bovine tuberculosis (Ben Kahla et al. 2011; Lamine-Khemiri et al. 2014; Djemal et al. 2017).

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To contribute to the knowledge of tuberculosis epidemiology and the genetics characterization of clinical species causing TB in Center Tunisia, we performed a retrospective microbiological analysis of Mycobacterium tuberculosis complex mycobacteria isolated in this region from 2008 to 2010 and 2014 to 2017. The current study was designed to identify the percentage of PTB and species causing it in the first project and to identify the molecular epidemiology of PTB and species lineage.

2. MATERIALS AND METHODS

2.1. Ethics

This study was approved by the local Medical Ethics Committee of the University Hospital Farhat Hached Sousse, Tunisia (approval number IRB 00008931; 2016).

2.2. Study Design

This retrospective study conducted in a coastal region of Central Tunisia incorporated all the Ziehl-Neelsen-positive cultures obtained from respiratory tract specimens and gastric juice specimens collected in patients suspected of pulmonary tuberculosis and submitted for the routine diagnosis of pulmonary tuberculosis in one university and tertiary care center Farhat Hacehd, Sousse, Tunisia. The characterization of these isolates was then made in the research unit MEPHI, IHU Méditerranée Infection, Marseille, France.

2.3. Study Population

This study included all the positive cultures of mycobacteria routinely made for the diagnosis of pulmonary tuberculosis from respiratory tract specimens and some related specimens (gastric juice, pleural fluid) collected in patients suspected of pulmonary tuberculosis, in Sousse in 2008-2010 and 2014-2017 (for a total of seven years). Respiratory tract specimens and gastric juice specimens routinely addressed to the Microbiology laboratory of University Hospital Center Farhat Hached, Sousse, Tunisia for the microbiological diagnosis of pulmonary tuberculosis were established by microscopic examination after Ziehl-Neelsen staining, decontamination by N-Acetyl-L-cysteine-sodium hydroxide and culture on Löwenstein-Jensen medium and Coletsos medium (BioRad, Roanne, France). All colonies were verified by Ziehl-Neelsen staining, and mycobacterial isolates were tentatively identified using conventional biochemical techniques and confirmed by GenoType molecular kits (Hain Life Science, Nehren, Germany) (Ahmed et al. 2017). Biochemical conventional techniques were based on catalase and peroxidase activities, the activity catalase was determined as follows, 100µL of the bacillary extract was added to 3mL of 5mM H2O2 in 50mM potassium phosphate (pH 7.0) and catalase activity was measured spectrophotometrically following the decrease in H2O2 concentration at a wavelength of 240nm to quantify the rate of decrease using an extinction coefficient of H2O2 at 240nm from 0.0435DO/mM.cm. Peroxidase activity was determined by the addition of 100µL of the 50mM potassium phosphate bacillary extract (pH 7.0) containing 0.1mM O-dianisidine and 23mM t-butyl hydroperoxide; absorbance at 460nm was monitored and velocity calculations were performed with an extinction coefficient of 11.3OD/mM.cm. Isolates were then inactivated by heating at 96°C for an hour (Djelouagj and Drancourt 2006) and stored at -80°C before being processed for molecular analyses in MEPHI, Marseille, France. Colonies which did not stain by Ziehl-Neelsen were not included in this study.

2.4. Procedures

DNA was extracted from colonies by using the EZ1 Advanced XL (Qiagen, Courtaboeuf, France) with EZ1 DNA Tissue Kit (Qiagen) according to the manufacturer's recommendations. All polymerase chain reaction (PCR) experiments incorporated sterile phosphate-buffered saline (PBS) as a negative control and DNA extracted from *M. tuberculosis* H37Rv as a positive control. In the first step, mycobacteria were identified by a standard PCR incorporating the rpoBF and rpoBR primer pair to amplify a 764-bp fragment of the *rpoB* gene (Adékambi et al. 2003) (Fig. 1). PCR products were purified and sequenced using the BigDye terminator 1.1 Cycle sequencing kit (Applied Biosystem, Courtaboeuf, France) and the ABI 3130 XL sequencer (Applied Biosystem). In a second step, the identification of mycobacteria as members of the M. tuberculosis complex was confirmed using quantitative real-time PCR (RT-PCR) targeting a 144-bp fragment of the internal transcribed spacer (ITS) (Fig. 2), which is present in all species of the *M. tuberculosis* complex (Bruijnesteijn Van Coppenraet et al. 2004). ITS was amplified using the ITSF and ITSR primer pair and hybridized with the ITSP probe as previously described (Bruijnesteijn Van Coppenraet, et al. 2004). In a third step, the identification at the species level within the *M. tuberculosis* complex was ensured by PCR-sequencing of the Exact Tandem Repeat D (ETRD) by using the ETRDF and ETRDR primer pair as previously described (Djelouagj et al. 2008). Finally, all the isolates identified as *M. tuberculosis sensu sticto* were genotyped in the *M. tuberculosis* Beijing family by detecting a 129-bp sequence in the IS*6110* insertion sequence, as previously described (Hillemann et al. 2006) and non-Beijing *M. tuberculosis* strains were further genotyped by detecting RD deletion regions RD239 and RD750 to differentiate *M. tuberculosis* lineages 1(Indo-Oceanic), lineage 3 (East-African-Indian) and lineage 4 (Euro-American) (Gagneux et al. 2006) (Fig. 3).

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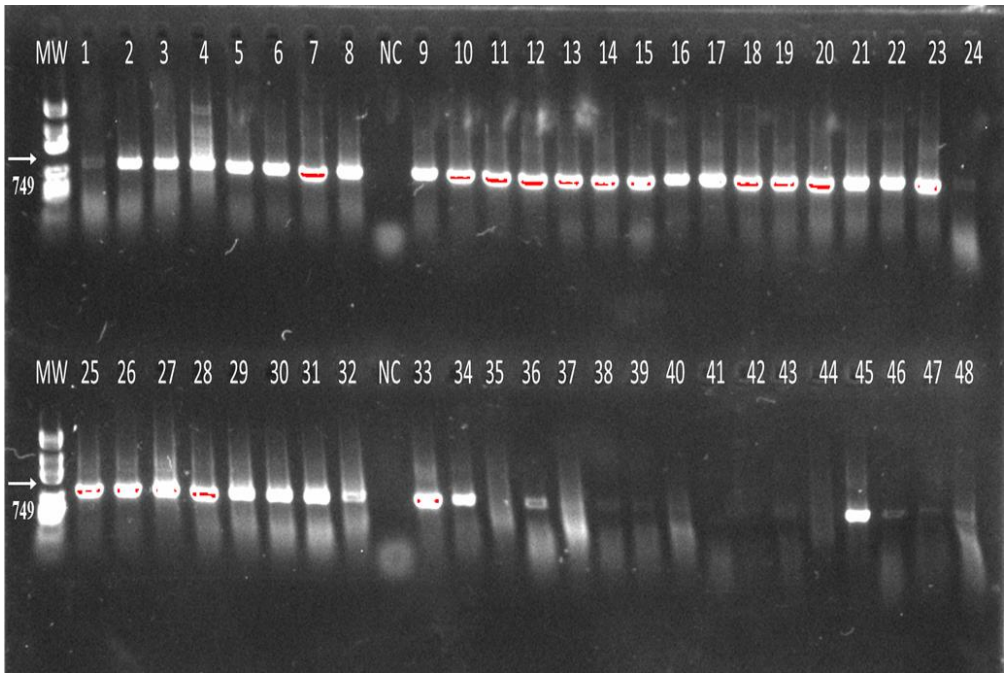

Fig. 1: PCR rpoB amplification (749bp). MW: Molecular weight (PGEM). NC: Negative control. Lane 1 to lane 48: Clinical samples.

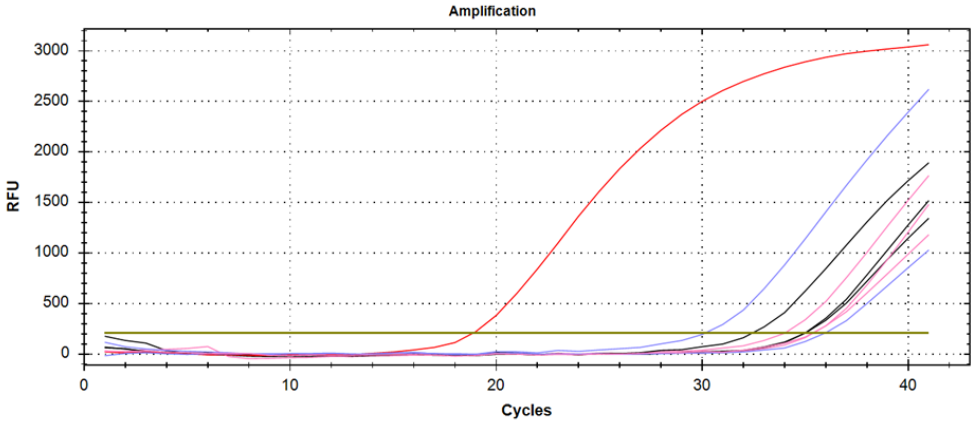

Fig. 2: Identification of *M. tuberculosis* ITS RT-PCR.

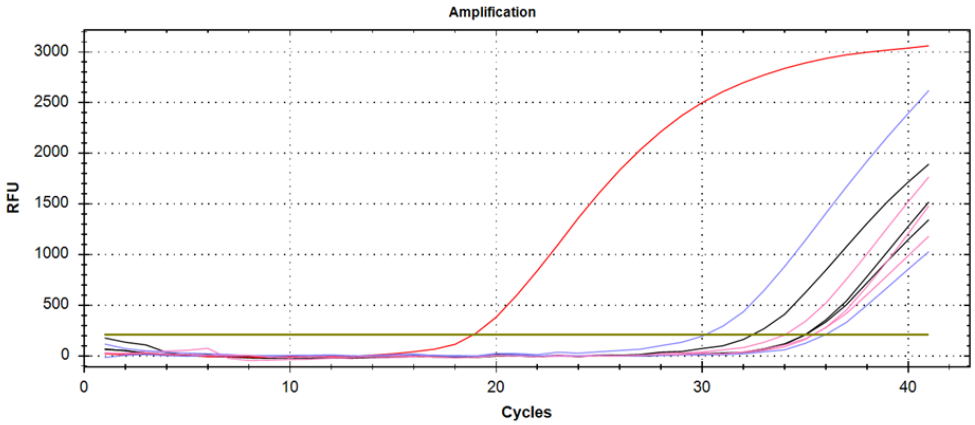

Fig. 3: Identification of *Mycobacterium tuberculosis* Beijing by RT-PCR. Redline: *M. tuberculosis* Beijing

2.5. Statistical Analysis

Statistical analysis was carried out using R tool to determine the linear regression and correlation between infection and increase in number of Tuberculosis Beijing (Fig. 4).

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3. RESULTS

A total of 302 cultures originating from respiratory tract specimens included 68 cultures made in 2008, 66 in 2009, 28 in 2010, 21 in 2014, 36 in 2015, 30 in 2016, and 47 in 2017. In addition, two pleural fluid specimens, one gastric juice specimen, and one bronchoalveolar wash were collected in 2015, and two gastric juice specimens were collected in 2017. In all PCR-based experiments, positive controls were positive, and negative controls remained negative. Sequencing a 764-bp *rpoB* fragment in 202 specimens yielded nontuberculous mycobacteria in four sputum specimens. Further, the RT-PCR ITS was positive in 295 specimens with average Ct value of 25.33 [range, 19.41-31.42] and remained negative in seven specimens (Table 1). In a further step, ERT-D sequencing yielded 294 *M. tuberculosis sensu stricto* isolates and four identified as *Mycobacterium bovis* BCG. At last, among 298 isolates identified as *M. tuberculosis* complex (positive for RT-PCR ITS and/or *rpoB* sequencing), 38 (12.6%) were genotyped as *M. tuberculosis* Beijing while the all the 168 non-Beijing *M. tuberculosis* isolates were identified as *M. tuberculosis* lineage 4 (Euro-American) (Table 1). Noteworthy, the prevalence of the Beijing genotype varied from 9.52% in 2014, 10.71% in 2010, 11.11% in 2015, 14.7% in 2008, 22.44% in 2017, and 23.33% in 2016. Statistical analyses indicated a linear correlation between the incidence of tuberculosis and the prevalence of the Beijing family isolates with a coefficient of linear regression R of 0.08 with a 95% confidence interval (-1.77, 1.94) and a nonsignificant p-value (P=0.91); meaning that an increase in the incidence of $10/10^5$ inhabitants corresponds to an increase in prevalence of 0.8% (Fig. 4).

Table 1: Summary of PCR and PCR-sequencing results

Year	Real-time PCR ITS				*rpo*B sequencing results		Positive RT-PCR *Beijing*
	Sample #	Positive	Negative	Average Ct	Positive	Negative	(%)
2008	68	66	2	31.02	59	9	10 (14.7)
2009	66	63	3	24.18	33	33	0 (0)
2010	28	27	1	30.61	12	16	3 (10.71)
2014	21	21	0	25.28	6	15	2 (9.52)
2015	40	39	1	24.71	33	7	5 (11.11)
2016	30	30	0	19.42	17	13	7 (23.33)
2017	49	49	0	22.09	42	7	11 (22.44)
Total	302	295	7	25.33	202	100	38 (12.58)

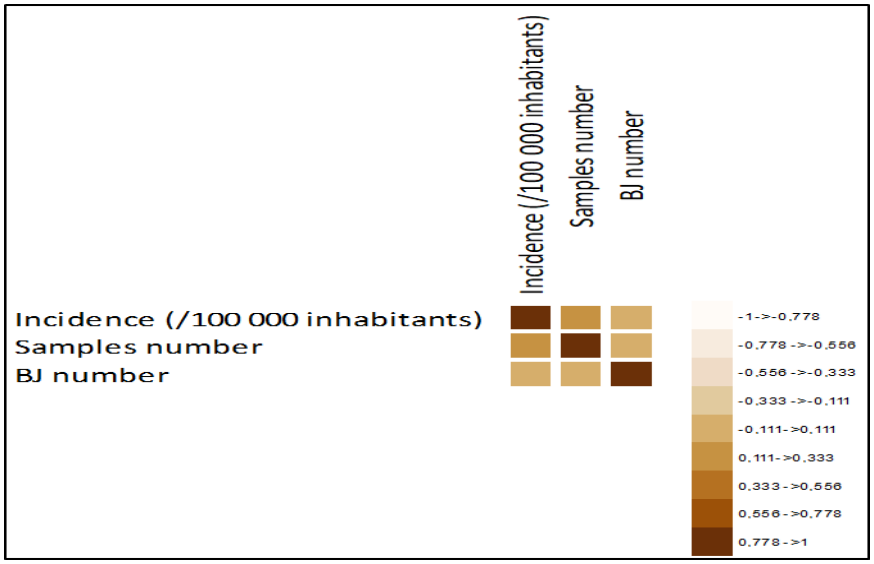

Fig. 4: Correlation between *M. tuberculosis* Beijing incidence and increases of infection.

4. DISCUSSION

Pulmonary tuberculosis remains a healthcare problem in Tunisia, where the epidemiological situation is globally contrasted with decreasing mortality but an increasing incidence of $24/10^5$ inhabitants per year in 2002 to $38/10^5$ inhabitants per year in 2016 (Ahmed et al. 2017; World Bank Group Data 2018). In order to gain an understanding of this situation in Tunisia, previous studies investigating the identification and resistance of isolates have been conducted at the regional scale, including eight studies conducted in North Tunisia (Chevrel-Dellagi et al. 1993; Hermans et al. 1995; Karboul et al. 2008; Namouchi et al. 2008; Soudani et al. 2010; Refai et al 2015; Meftahi et al. 2016; Dekhil et al. 2016), two studies in the South (Smaoui et al. 2015; Siala et al. 2017) and only two in Central Tunisia (Kahla et al. 2011; Ben Kahla et al. 2011). Therefore, we performed a retrospective study in

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the Center Tunisia region.

Here, all the 302 cultures established from patients suspected of having pulmonary tuberculosis in Central Tunisia were subjected to a polyphasic molecular approach of identification. The data reported here were authenticated by incorporating both negative and positive controls in every PCR-based experiment, and the fact that the identification of mycobacteria within the M. tuberculosis complex was made by two different methods, which yielded identical results. As for isolates identified in the M. tuberculosis complex, we found the ETRD system to be efficient in identification at the species level, as previously reported (Djelouadji et al. 2008; Coitinho et al. 2012). This approach yielded *M. tuberculosis* sensu stricto in most isolates, whereas four isolates were identified as *M. bovis* BCG. Three isolates were made in 2017 from three immunocompromised children who developed BCGitis following BCG vaccination at birth, and the interpretation of a fourth isolate made in 2008 was not possible due to a lack of clinical information.

While majority of *M. tuberculosis* isolates were genotyped as lineage 4, the prevalent lineage currently reported in Tunisia (Stucki et al. 2016), we also identified a stepwise increase in the prevalence of the Beijing family of *M. tuberculosis*, which correlates with the increasing incidence of pulmonary tuberculosis in Tunisia. The origin of this phenomenon warrants further study.

5. CONCLUSION AND PERSPECTIVES

The herein report of four non-tuberculous mycobacteria from the respiratory tract specimens collected from Tunisian patients suspected of pulmonary tuberculosis underscores the necessity of accurate identification of mycobacterial isolates to help in the medical management of such patients appropriately. Moreover, data herein reported suggest that the unique epidemiology of pulmonary tuberculosis in central Tunisia and Tunisia at large is driven by the emergence of the Beijing family of *M. tuberculosis* in the country.

Competing Interests: The authors declare that they have no competing interests.

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Author's Contributions

MD: Conceived the idea, tailored the experiments, verified data, and drafted the manuscript. ABHH: Performed the experiments and drafted the manuscript. MM and JB: Collected the isolates, performed first-line identification, and revised the manuscript. JS: Performed genotyping. All authors approved the revised manuscript.

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REFERENCES

Adékambi T, Colson P and Drancourt M, 2003. rpoB-based identification of nonpigmented and late-pigmenting rapidly growing mycobacteria. Journal of Clinical Microbiology, 41(12): 5699-5708. https://doi.org/10.1128/JCM.41.12.5699-5708.2003

Ahmed BHH, Marzouk M, Mohamed D and Jalel B, 2017. Disseminated *Bacille Calmette-Guérin* infection in immunodeficient infants: Report of two cases. Archives of Pediatric Infectious Diseases 13(5): 799–801. https://doi.org/10.5812/pedinfect.37490

Ben Hadj Hassine A, Marzouk M, Dhaou M and Boukadida J, 2017. Glutaraldehyde test for the rapid diagnosis of pulmonary and extra-pulmonary tuberculosis in an area with high tuberculosis incidence. Memorias do Instituto Oswaldo Cruz 112(11) : 779–784. https://doi.org/10.1590/0074-02760160541

Ben Kahla I, Boschiroli MI, Souissi F, Cherif N, Benzarti M, Boukadida J and Hammami S, 2011. Isolation and molecular characterisation of Mycobacterium bovis from raw milk in Tunisia. African Health Sciences 11(Pt1): S2–S5. https://doi.org/10.4314/ahs.v11i3.70032

Ben Kahla I, Marzouk M, Henry M, Bedotto M, Cohen-Bacrie S, Ben Selma W, Boukadida J, and Drancourt M, 2011. Molecular characterisation of isoniazid- and rifampicin-resistant Mycobacterium tuberculosis in Central Tunisia. The international journal of tuberculosis and lung disease: the official journal of the International Union against Tuberculosis and Lung Disease 12(4): 1685-1688. https://doi.org/10.5588/ijtld.11.0185

Bruijnesteijn van Coppenraet ES, Lindeboom JA, Prins, JM, Peeters MF, Claas ECJ and Kuijper EJ, 2004. Real-Time PCR assay using fine-needle aspirates and tissue biopsy specimens for rapid diagnosis of mycobacterial lymphadenitis in children. Journal of Clinical Microbiology 42(6): 2644-2650. https://doi.org/10.1128/jcm.42.6.2644-2650.2004

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Chevrel-Dellagi D, Abderrahman A, Haltiti R, Koubaji H, Gicquel B and K Dellagi, 1993. Large-scale DNA fingerprinting of Mycobacterium tuberculosis strains as a tool for epidemiological studies of tuberculosis. Journal of Clinical Microbiology 31(9): 2446-50. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC265776/

Coitinho C, Greif G, Robello C, van Ingen J and Rivas C, 2012. Identification of Mycobacterium tuberculosis complex by polymerase chain reaction of Exact Tandem Repeat-D fragment from mycobacterial cultures. International Journal of Mycobacteriology 1(3): 146-148. https://doi.org/10.1016/j.ijmyco.2012.07.002

Dekhil N, Meftahi N, Mhenni B, Ben Fraj S, Haltiti R, Belhaj S, and Mardassi H, 2016. MDR-TB Outbreak among HIV-Negative Tunisian Patients followed during 11 Years. PLoS ONE 11(4): e0153983. https://doi.org/10.1371/journal.pone.0153983

Djelouagj Z and Drancourt M, 2006. Inactivation of cultured Mycobacterium tuberculosis organisms prior to DNA extraction. Journal of Clinical Microbiology 44 (4): 1594-1595. https://doi.org/10.1128/JCM.44.4.1594-1595.2006

Djemal S, Siala M, Smaoui S, Kammoun S, Marouane C, Bezos J, Messadi-Akrout F, Romero B and Gdoura R, 2017. Genetic diversity assessment of Tunisian Mycobacterium bovis population isolated from cattle. BMC Veterinary Research 13: 393. https://doi.org/10.1186/s12917-017-1314-y

Gagneux S, DeRiemer K, Van T, Kato-Maeda M, de Jong BC, Narayanan S, Nicol M, Niemann S, Kremer K, Gutierrez MC, Hilty M, Hopewell PC and Small PM, 2006. Variable host-pathogen compatibility in Mycobacterium tuberculosis. Proceedings of the National Academy of Sciences of the United States of America 103(8): 2869-2873. https://doi.org/10.1073/pnas.0511240103

Hermans PW, Messadi F, Guebrexabher H, van Soolingen D, de Haas Pe, Heersma H, de Neeling H, Ayoub A, Portaels F and Frommel D, 1995. Analysis of the population structure of Mycobacterium tuberculosis in Ethiopia, Tunisia, and The Netherlands: usefulness of DNA typing for global tuberculosis epidemiology. The Journal of Infectious Diseases 171(6): 1504-1513. https://doi.org/10.1093/infdis/171.6.1504

Hillemann D, Warren R, Kubica T, Rüsch-Gerdes S and Stefan NiemannS, 2006. Rapid Detection of Mycobacterium tuberculosis Beijing Genotype Strains by Real-Time PCR. Journal of Clinical Microbiology 44(2): 302-306. https://doi.org/10.1128/JCM.44.2.302-306.2006

Kahla IB, Henry M, Boukadida J and Drancourt M, 2011. Pyrosequencing assay for rapid identification of Mycobacterium tuberculosis complex species. BMC Research Notes 19(4): 423. https://doi.org/10.1186/1756-0500-4-423

Karboul A, Mazza A, Gey van Pittius N, Ho JL, Brousseau R and Mardassi H, 2008. Frequent homologous recombination events in Mycobacterium tuberculosis PE/PPE multigene families: potential role in antigenic variability. Journal of Bacteriology 190(23): 7838-7846. https://doi.org/10.1128/JB.00827-08

Lamine-Khemiri H, Martínez R, García-Jiménez WI, Benítez-Medina JM, Cortés M, Hurtado I, Abassi MS, Khazri I, Benzarti M and Hermoso-de-Mendoza J, 2014. Genotypic characterization by spoligotyping and VNTR typing of Mycobacterium bovis and Mycobacterium caprae isolates from cattle of Tunisia. Tropical Animal Health and Production 46(2): 305–311. https://doi.org/10.1007/s11250-013-0488-y

Meftahi N, Namouchi A, Mhenni B, Brandis G, Hughes D and Mardassi H, 2016. Evidence for the critical role of a secondary site rpoB mutation in the compensatory evolution and successful transmission of an MDR tuberculosis outbreak strain. The Journal of Antimicrobial Chemotherapy 71(2): 324-332. https://doi.org/10.1093/jac/dkv345

Namouchi A, Karboul A, Mhenni B, Khabouchi N, Haltiti R, Ben Hassine R, Louzir B, Chabbou A and Mardassi H, 2008. Genetic profiling of Mycobacterium tuberculosis in Tunisia: predominance and evidence for the establishment of a few genotypes. Journal of Medical Microbiology 57(Pt 7): 864–872. https://doi.org/10.1099/jmm.0.47483-0

Refai A, Haoues M, Othman H, Barbouche Mr, Moua P, Bondon A, Mouret L, Srairi-Abid N and Essafi M, 2015. Two distinct conformational states of Mycobacterium tuberculosis virulent factor early secreted antigenic target 6 kDa are behind the discrepancy around its biological functions. The FEBS Journal 282(21): 4114-4129. https://doi.org/10.1111/febs.13408

Siala M, Smaoui S, Taktak W, Hachicha S, Ghorbel A, Marouane C, and Kammoun S, 2017. First-time detection and identification of the Mycobacterium tuberculosis complex members in extrapulmonary tuberculosis clinical samples in south Tunisia by a single tube tetraplex real-time PCR assay. PLoS Neglected Tropical Diseases 11(5): e0005572. https://doi.org/10.1371/journal.pntd.0005572

Smaoui S, Mezghanni MA, Hammami B, Zalila N, Marouane C, Kammoun S, Ghorbel A, Ben Jemaa M and Messadi-Akrout F, 2015. Tuberculosis lymphadenitis in a southeastern region in Tunisia: Epidemiology, clinical features, diagnosis and treatment. International Journal of Mycobacteriology 4(3): 196-201. https://doi.org/10.1016/j.ijmyco.2015.04.004

Soudani A, Hadjfredj S, Zribi M, Messaadi F, Messaoud T, Masmoudi A, Zribi M and Fendri C, 2011. Genotypic and phenotypic characteristics of tunisian isoniazid-resistant Mycobacterium tuberculosis strains. Journal of Microbiology (Seoul, Korea) 40: 413–417. https://doi.org/10.1007/s12275-011-0268-1

Soudani A, Hadjfredj S, Zribi M, Messaoud T, Masmoudi A, Majed B and Fendri C, 2010. First report of molecular characterization of fluoroquinolone-resistant Mycobacterium tuberculosis isolates from a Tunisian hospital. Clinical Microbiology and Infection 6(9): 1454-1457. https://doi.org/10.1111/j.1469-0691.2009.03087.x

Stucki D, Brites D, Jeljeli L, Coscolla M, Liu Q, Trauner A, Fenner L, Rutaihwa L, Borrell S, Luo T, Gao Q, Kato-Maeda M, Ballif M, Egger M, Macedo R, Mardassi H, Moreno M, Tudo Vilanova G, Fyfe J, Globan M, Thomas J, Jamieson F, Guthrie JL, Asante-Poku A, Yeboah-Manu D, Wilkinson R, Skenders G, Hoffner S, Bakonyte D, Stakenas P, Diel R, Crudu V, Moldovan O, Al-Hajoj S, Otero L, Barletta F, Jane Carter E, Diero L, Supply P, Comas I, Niemann S and Gagneux S, 2016. Mycobacterium tuberculosis lineage 4 comprises globally distributed and geographically restricted sublineages. Nature Genetics 48(12): 1535–1543. https://doi.org/10.1038/ng.3704

World Bank Open Data, 2024. World Bank Open Data: Free and open access to global development data. https://data.worldbank.org

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