GENETIC DIVERSITY OF *Mycobacterium tuberculosis* IN JOS, NIGERIA

Agatha Ani¹, Torbjørn Bruvik², Yetunde Okoh¹, Patricia Agaba³, Oche Agbaji³, John Idoko³, and Ulf R Dahle²*

¹ Department of Medical Microbiology, Faculty of Medical Sciences, University of Jos, Nigeria

² Division of Infectious Disease Control, Norwegian Institute of Public Health, Oslo, Norway,

³ APIN Centre Jos University Teaching Hospital, Jos, Nigeria

*Corresponding author

Correspondence to: Ulf R. Dahle, Division of Infectious Disease Control, Norwegian Institute of Public Health, 0403 Oslo, Norway. Tel: (+47) 23076365, Fax (+47)23076518, e-mail: ulf.dahle@fhi.no

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Abstract

Background

Nigeria has a high tuberculosis incidence, and genotyping studies of *Mycobacterium tuberculosis* in the country are necessary in order to improve our understanding of the epidemic.

Methods

Genotyping of bacterial DNA was performed by spoligotyping. The SpolDB4 database and the model-based program ‘spotclust’ were used to assign isolates to families, subfamilies and variants.

Results

A total of 111 pulmonary isolates from consecutive tuberculosis patients in the city of Jos, Plateau State, Nigeria were spoligotyped. The findings were highly homogenous with little genetic variation, and were assigned to genetic lineages based on their spoligopatterns. A total of 84 (76%) of the isolates belonged to the Latin American Mediterranean (LAM) family. Of these were 78 isolates assigned to the LAM10 lineage. Among these, 59 carried identical spoligopatterns. Drug resistance data were also obtained for most isolates, and could not be correlated to the genetic clustering of the isolates.
Conclusions

The dominance of few *M. tuberculosis* lineages indicate a high rate of transmission, high levels of synonymously import or a highly conserved genotype. It remains to be confirmed whether the large cluster of identical LAM10 represent an outbreak. Spoligotyping is useful to gain an overall understanding of the local TB epidemic. This study demonstrates that the extensive TB epidemic in Jos, Nigeria is caused by a few successful *M. tuberculosis* families, dominated by the LAM10 family.
Background

Nigeria ranks fifth among the world’s high-burden countries, with a number of tuberculosis (TB) cases of 450,000. This puts the tuberculosis (TB) incidence at 311/100,000 and the rate of new sputum smear positive disease is approximately 137/100,000 [1].

Points of concern include the proportion of patients lost to follow-up, diagnostic delay, low case detection rate and the continuing high prevalence of HIV. The high case rate in many African countries contributed to a rise of the global TB incidence of 1% in 2003, despite stable or declining rates in the rest of the world. but the incidence rate in Nigeria declined by 1.3% between 2005 and 2006 [1]. In order to improve our understanding of the TB epidemic in this high-incidence country, the current study included \textit{M. tuberculosis} strains collected in Jos during three intervals of 2008. Currently no laboratory in Nigeria offers spoligotyping services. Spoligotyping is a PCR-based fingerprinting method that detects the presence or absence of 43 defined spacers situated between short direct repeat (DR) sequences in the genomes of members of the \textit{M. tuberculosis} complex [2]. Important advantages of spoligotyping are that it is cheap, easy to perform and fast. In addition, it has been demonstrated that the results are highly reproducible [3]. Unique to spoligotyping results are tools like the SpolDB4 database [4] and the web-based computer algorithm ‘Spotclust’ [5] that can be used to assign new isolates to families, subfamilies and variants (SpolDB4 only). SpolDB4 is the largest and most up to date available global database for spoligotypes. For previously not reported spoligopatterns, the ‘Spotclust’ database is a good additional tool in that it can assign these patterns to families by using a computer algorithm based on studies of SpolDB3. The results from local studies can thus be analyzed and compared to the global \textit{M. tuberculosis} population. This may
help us better understand the world-wide spread of common *M. tuberculosis* families and subfamilies. In the current ongoing study we describe the diversity of *M. tuberculosis* isolates from Jos, Nigeria, based on spoligotyping, and identify the families and subfamilies responsible for the current persistence and spread of TB in this high-incidence community.
Results

Genetic diversity and family assignment

The 111 analyzed isolates gave 30 different spoligopatterns resulting in an overall diversity of 27%: 17 spoligopatterns occurred only once and 1 pattern comprised 65% of the isolates (table 1). All patterns had been described previously [4]. Family assignment by use of the SpolDB4 database and ‘Spotclust’ showed that 76% of the isolates belonged to the Latin American Mediterranean (LAM) family, of these were 93% assigned to the LAM 10 family. A total of 9% of the isolates were assigned to the Haarlem family, 5% to the X family, 4% to the T family and F family and 2% to the EAI family. The LAM10 thus clearly dominate the TB epidemic in Jos (Figure 1). The main families were well distinguished and a low diversity within the population was observed.

The rate of diversity (number of spoligotypes divided by the number of isolates) within the LAM family was very low (17%). This may indicate that the LAM family is well adapted to spread, highly conserved, or recently transmitted within this community. The M. tuberculosis population in Jos was comparable to that described in previous studies from Cameroon and Burkina Faso [6,7]. In Dar es Salaam, Tanzania, the overall genetic diversity was 52% [8]. Thus, the diversity in high-incidence countries varies greatly and may be difficult to estimate without molecular epidemiological studies.
Discussion

The current study demonstrated that most isolates had at least one other closely related isolate in Jos. The TB epidemic thus appeared to result from a gradually evolving \textit{M. tuberculosis} population rather than recently imported strains. A study conducted in the West province of Cameroon found that 193 of 413 \textit{M. tuberculosis} isolates belong to the Cameroon family (LAM10-CAM) \cite{6}, and in Harare, Zimbabwe, 68 of 214 isolates are LAM11-ZWE variants \cite{9}. Of the 111 isolates in this study, 78 were LAM10 and no LAM11 were observed. Although other lineages are found in other countries, these findings indicate that various LAM sub-families dominate in various regions of Africa and that the TB epidemics are multiple and local. Since lineages that are abundant in other African countries (such as the LAM11) were absent in the current study, and that the current population was highly clonal, we believe that the TB epidemic in Jos, Nigeria is local and well established. The success of the LAM family in particular in this community is intriguing. The highly prevalent LAM10 family in this study may indicate that the family is spreading rapidly, but could also reflect a slow evolution of the DR region. The South American and West-African success of the LAM family suggest a possible co-evolution between specific \textit{M. tuberculosis} families and host population. The molecular basis of this remains to be elucidated but this pattern might reflect the last centuries’ transport and interaction between these continents and the genetic relatedness between Western Africa and the Americas.
By using spoligotyping one can identify outbreaks, support community-based contact tracing, describe the diversity of a *M. tuberculosis* population, and compare this population to that in other parts of the world [4,10,11]. Implementation of spoligotyping as a routine method for molecular epidemiological studies of *M. tuberculosis* isolates, appear to represent a valuable investment in many high-incidence countries.
Conclusions

Spoligotyping was useful to gain an overall understanding of the local TB epidemic in Jos, Nigeria. This study demonstrated that the extensive TB epidemic in this area was caused by one successful *M. tuberculosis* family, dominated by the LAM10 subfamily. Import of new strains from neighbouring countries represents a minor problem and the spread of TB in this area appear to be locally restricted.
Methods

Settings

Ethical clearance was granted respectively by The Jos University Teaching Hospital and Plateau State Hospital ethical committees. The study was descriptive of a bacterial collection and contained no material of human origin. Personal data were removed from all bacterial cultures to protect the anonymity of the patients. Patient informed consent was therefore not obtained.

*M. tuberculosis* isolates were isolated on Löwenstein Jensen medium at the Faculty of Medical Microbiology, University of Jos, Nigeria. The strains were isolated from patients with newly diagnosed smear-positive cases of pulmonary tuberculosis. These were diagnosed consecutively during two periods in 2008. Cultures were heat inactivated at $80^\circ$ for 20 minutes. A total of 111 heat-killed *M. tuberculosis* strains were sent to the National Reference Laboratory for Mycobacteria at the Norwegian Institute of Public Health (NIPH). Upon arrival, DNA was extracted and spoligotyping performed as described elsewhere [2].
DNA extraction and spoligotyping

DNA was extracted and spoligotyping was performed according to Kamerbeek et al. [2].

Family assignment

The obtained spoligopatterns were first compared to the SpolDB4 database and assigned to families and subfamilies [4]. Second, in order to assign names to the isolates not found in the SpolDB4 database, the spoligopatterns were analyzed with ‘Spotclust’, using a mixture model built on the SpolDB3 database [5]. This model takes into account knowledge of the evolution of the DR region and assigns spoligopatterns to families and subfamilies.
**Competing interests**

The authors declare that they have no competing interests in the subject matter.

**Authors’ contributions**

AA contributed to conception and design of the study, laboratory work and data analysis. TB, YO and PA participated in the laboratory work and data analyses. JI participated in the design of the study and the data analyses. UD conceived the study, supervised and participated in the laboratory work and data analyses. All authors contributed in the writing of the article, read and approved the final manuscript.

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**Legends**

Genetic similarity and spoligopattern of *Mycobacterium tuberculosis* isolates from Jos in Nigeria. The population demonstrates a high degree of homogeneity and dominance of LAM10.
Reference List


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