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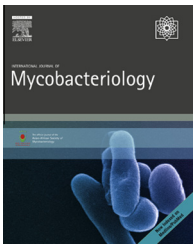

Short Communication

Genetic diversity of *Mycobacterium tuberculosis* complex strains isolated from patients with pulmonary tuberculosis in Anambra State, Nigeria

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ARTICLE INFO

Article history:
Received 8 May 2015
Received in revised form
10 June 2015
Accepted 11 June 2015
Available online 29 July 2015

Keywords:
Exact tandem repeat
Mycobacterium tuberculosis
Nigeria
Spoligotyping
Tuberculosis
Variable number of tandem repeats

ABSTRACT

In this study, we analyzed *Mycobacterium tuberculosis* complex (MTC) genetic diversity in Anambra State, Nigeria based on spoligotyping followed by 5-loci exact tandem repeats (ETRs). Spoligotyping of 180 MTC strains isolated in 2009–2011 from pulmonary tuberculosis (TB) patients led to a total of 31 distinct patterns. A comparison with the SITVIT2 international database showed that all the 31 patterns could be classified as Shared-types (SITs) in this database; briefly, 26/31 SITs (n = 174 isolates) matched a preexisting shared-type in the database, whereas 5/31 SITs (*n* = 6 isolates) were newly created due to 2 or more strains belonging to an identical new pattern within this study (SIT3396) or after a match with an orphan in the database (SIT3397, SIT3398, SIT3399 and SIT3400). A total of 18/31 SITs containing 167 or 92.8% isolates were clustered within this study (2–89 isolates per cluster) while 13/31 SITs contained unique strains. Using VNTR typing, a total of 36 distinct patterns were identified; 27 patterns (*n* = 157 isolates) matched a pattern already reported in the SITVIT2 database. Combination of both the methods generated 47 combined patterns for the 180 strains: 17 belonged to clustered isolates (*n* = 127 isolates or 70.5%) while 30 corresponded to as many unique strains (note 23 strains could not be typed using 5-loci ETRs). No correlation was found between the spoligotyping pattern and the HIV status of the patient or drug sensitivity of the strain. This study showed that the LAM10-CAM prototype SIT61 accounted for highest number of isolates (*n* = 89) in Anambra State, showing its relative contribution to the TB burden in the study.

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Peer review under responsibility of Asian African Society for Mycobacteriology.
http://dx.doi.org/10.1016/j.ijmyco.2015.06.008

Introduction

Nigeria, with a population of over 150 million, is among the high-tuberculosis (TB)-burden countries and ranks 13th in the world [1]. Multiple-drug-resistant TB (MDR-TB) is another problem, and in a recent study, it has been found that as much as 8% of all cultured specimens were MDR-TB positive in three states in Nigeria [2]. The information available on the incidence, drug susceptibility, and genotyping of the *Mycobacterium tuberculosis* complex (MTC) in Nigeria is limited [3–6]. Additional data are needed to explore the population structure of strains of MTC to identify specific endemic strains in the study area; monitor transmission dynamics to link outbreak cases in communities, hospitals, or institutions; and for better treatment.

Many molecular-typing techniques have been used to differentiate strains of MTC involved in TB infection, among which the spoligotyping method based on the polymorphism of the direct repeat locus is a widely used first-line typing method [2,6]. However, when used alone, the lower discriminatory power of spoligotyping requires that it is ideally used in association with 12, 15, or 24-loci mycobacterial interspersed repetitive unit–variable number of tandem repeats (MIRU–VNTRs) for *M. tuberculosis* molecular epidemiology, or at minima in association with a more convenient five-loci exact tandem repeats (ETRs, [7]) that have been successfully used to improve the potential of spoligotyping for studying the genetic diversity of TB [7–9]. The present study constitutes a first attempt to describe the genetic population structure of MTC circulating in Anambra State, Nigeria using spoligotyping and five-loci ETRs.

Materials and methods

Setting, clinical isolates, and molecular characterization

The study was conducted among patients between the ages of 10 years and 82 years with pulmonary TB attending Nnamdi Azikiwe University Teaching Hospital and different peripheral DOTS clinics in Anambra State during the period 2009–2011. Data regarding the patients' gender, human-immunodeficiency-virus (HIV) status, and age were collected. MTC strains were isolated and identified from 550 sputum samples of suspected TB patients after smear microscopy by the Ziehl–Neelsen method at Nnamdi Azikiwe University Teaching Hospital, Nnewi, and cultured on Löwenstein–Jensen medium at Zankli TB laboratory, Abuja. DNA was extracted using the classical cetyl-trimethyl-ammonium-bromide method as described previously [8,10], and sent to the University of Hertfordshire, Hatfield, England for molecular typing. Spoligotyping was performed using a commercially available kit (Ocimum Biosolutions, Hyderabad, India), following the manufacturer's instructions, and previously described methodology [11], shown to be useful to study the transmission of *M. tuberculosis* [12]. Five-loci ETR (A, B, C, D, and E) typing was performed, as described by Frothingham and Meeker-O'Connell [7]. The exact number of tandem repeats at each locus was analyzed for each strain using polymerase chain reaction.

Database comparison

The identified spoligotypes and five-loci ETR patterns were analyzed using the BioNumerics software (BioSystematica), and compared with the SITVIT2 proprietary database of the Institut Pasteur de Guadeloupe, which is an updated in-house version of the recently released SITVITWEB database [13], available online at http://www.pasteur-guadeloupe.fr:8081/SITVIT_ONLINE/. In this database, spoligotype international type (SIT) and VNTR international type (VIT) designate spoligotype and five-loci ETR patterns shared by two or more patient isolates, as opposed to "orphan," which designates patterns reported for a single isolate. Major phylogenetic clades were assigned according to the signatures provided in the database defining 62 genetic lineages/sublineages. These include various MTC members, such *as Mycobacterium bovis, Mycobacterium caprae, Mycobacterium microti, Mycobacterium canettii, Mycobacterium pinnipedii*, and *Mycobacterium africanum*, as well as rules defining major lineages/sublineages for *M. tuberculosis* sensu stricto. These include the Beijing clade, the Central-Asian clade and two sublineages, the East-African–Indian clade and nine sublineages, the Haarlem clade and three sublineages, the Latin-American–Mediterranean (LAM) clade and 12 sublineages (note that two sublineages, LAM7-TUR and LAM10–CAM, were reclassified as Turkey and Cameroon lineages), the ancestral "Manu" family and three sublineages, the S clade, the IS6110-low-banding X clade and three sublineages, and an ill-defined T clade with five sublineages.

The description of predominant clusters in this study (four or more isolates) and their worldwide distribution was studied in function of their reported numbers in various macrogeographical regions in the SITVIT2 database (reported for regions with more than 3% of a given shared type). The definition of macrogeographical regions and subregions (http://unstats.un.org/unsd/methods/m49/m49regin.htm) was according to the United Nations scheme (regions: AFRI [Africa], AMER [Americas], ASIA [Asia], EURO [Europe], and OCE [Oceania], subdivided in E [eastern], M [middle], C [central], N [northern], S [southern], SE [southeastern], and W [western]). Note that, in this scheme, CARIB (Caribbean) belongs to Americas, while Oceania is subdivided in four subregions: AUST (Australasia), MEL (Melanesia), MIC (Micronesia), and POLY (Polynesia). Furthermore, Russia was attributed a new subregion by itself (Northern Asia), instead of including it among the rest of Eastern Europe, reflecting its geographical localization, as well as due to the similarity of specific TB genotypes circulating in Russia (a majority of Beijing genotypes) with those prevalent in Central, Eastern, and Southeastern Asia. Finally, the three-letter country codes were according to http://en.wikipedia.org/wiki/ISO_3166-1_alpha-3.

Ethical considerations

An ethical clearance was granted by the hospital ethical committee, and informed consent was obtained from each patient.