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# Reduced transmission of *Mycobacterium africanum* compared to *Mycobacterium tuberculosis* in urban West Africa

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ABSTRACT

*Objective:* Understanding transmission dynamics is useful for tuberculosis (TB) control. A population-based molecular epidemiological study was conducted to determine TB transmission in Ghana.
*Methods:* *Mycobacterium tuberculosis* complex (MTBC) isolates obtained from prospectively sampled pulmonary TB patients between July 2012 and December 2015 were characterized using spoligotyping and standard 15-locus mycobacterial interspersed repetitive unit variable number tandem repeat (MIRU-VNTR) typing for transmission studies.
*Results:* Out of 2309 MTBC isolates, 1082 (46.9%) unique cases were identified, with 1227 (53.1%) isolates belonging to one of 276 clusters. The recent TB transmission rate was estimated to be 41.2%. Whereas TB strains of lineage 4 belonging to *M. tuberculosis* showed a high recent transmission rate (44.9%), reduced recent transmission rates were found for lineages of *Mycobacterium africanum* (lineage 5, 31.8%; lineage 6, 24.7%).
*Conclusions:* The study findings indicate high recent TB transmission, suggesting the occurrence of unsuspected outbreaks in Ghana. The observed reduced transmission rate of *M. africanum* suggests other factor(s) (host/environmental) may be responsible for its continuous presence in West Africa.


## Introduction

Tuberculosis (TB) is a global health emergency; in 2016 an estimated 10.4 million people got sick, while 1.7 million died of TB (WHO, 2017). In 1993, the World Health Organization (WHO) declared TB a global health emergency and called for more efforts and resources to fight TB. Due largely to the inefficacy of the bacillus Calmette–Guérin (BCG) vaccine against pulmonary TB in adults, the current TB control strategy relies on case detection and treatment under the directly observed therapy short course (DOTs) strategy. The conventional indicators used to assess national control programs under this strategy focus on the proportion of cases that are cured at the end of treatment or whose sputum microscopy becomes negative after the first 2 months of treatment. Such indicators ignore equally important aspects of TB control, which include the duration of infectivity, the frequency of reactivation, and the risk of progression among the infected contacts, as well as the proportion of TB due to recent transmission.

Understanding transmission dynamics will contribute to knowledge on factors that enhance the spread of the disease, which is useful for developing preventive interventions. Molecular epidemiological studies have been very useful in a number of countries, identifying populations at risk and areas of high transmission, as well as providing much understanding on the prevalence of different *Mycobacterium tuberculosis* complex (MTBC) strains with varied virulence and drug resistance rates

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(Anderson et al., 2014; Malm et al., 2017; Seto et al., 2017; Varghese et al., 2013; Walker et al., 2014; Yang et al., 2016). These studies have shown that the dynamics of TB transmission vary greatly geographically. Even though Africa harbors a large proportion of the global TB cases, with a current incidence of 254 per 100 000 population (WHO, 2017), population-based molecular epidemiological studies needed to understand transmission patterns are rare. The few studies conducted have not been population-based and have lacked an in-depth analysis of the transmission dynamics of MTBC strains belonging to different lineages (Asante-Poku et al., 2016; Glynn et al., 2010; Mulenga et al., 2010).

The molecular typing tools – spacer oligonucleotide typing (spoligotyping) and mycobacterial interspersed repetitive unit variable number tandem repeat (MIRU-VNTR) typing – have been used successfully for strain differentiation in TB transmission studies due to their combined high discriminatory power and reproducibility; furthermore, in combination with epidemiological data, they have been used for the detection of recent TB transmission and outbreaks (Anderson et al., 2014; Barnes and Cave, 2003; Maguire et al., 2002; Surie et al., 2017; Varghese et al., 2013). Currently, the high cost and expertise needed for whole genome sequencing and analysis have precluded its use in population-based studies, and considering capacity building in a low-resource setting like Ghana, spoligotyping and MIRU-VNTR typing remain good alternatives.

TB in humans is caused mainly by *Mycobacterium tuberculosis* sensu stricto (MTBss) and *Mycobacterium africanum* (MAF), which are further divided into seven lineages: MTBss lineages 1–4 and 7 (L1–L4 and L7); MAF lineages 5 and 6 (L5 and L6) (Blouin et al., 2012; de Jong et al., 2010). While MTBss is distributed globally, MAF is restricted to West Africa, where it is responsible for up to 50% of TB cases (Gagneux and Small, 2007). Nevertheless, reports mainly from the Gambia where L6 is prevalent, suggest MAF is attenuated compared to MTBss, hence could be outcompeted by MTBss (de Jong et al., 2010, 2008; Kallenius et al., 1999). However, an 8-year study recently conducted in Ghana found the prevalence of MAF to be fairly constant at approximately 20%, indicating that MAF and MTBss may be transmitted equally (Yeboah-Manu et al., 2016). The objective of this study was to determine the transmission dynamics of TB caused by MTBss and MAF in Ghana.

## Methods

### *Study design and population*

This study was a population-based prospective study in which sputum samples were collected from consecutive clinically diagnosed pulmonary TB patients reporting to 12 selected health facilities within an urban setting (Accra Metropolitan Assembly (AMA)) and the rural setting of East Mamprusi District (MamE) (Supplementary material, Figure S1). The study was conducted from July 2012 to December 2015. A pulmonary TB case was defined as an individual with a case of TB that was confirmed both clinically and bacteriologically. Detailed demographic and epidemiological data were obtained from consented participants.

### *Mycobacterial isolation, species identification, and drug susceptibility testing*

The sputum samples were decontaminated and cultured on Lowenstein–Jensen medium to obtain mycobacterial isolates. These isolates were confirmed as MTBC by detecting the MTBC-specific insertion sequence IS*6110* using PCR (Yeboah-Manu et al., 2001). In vitro drug susceptibility to isoniazid and rifampicin were determined using either the microplate Alamar Blue cell viability assay, as described elsewhere (Otchere et al., 2016), and/or the GenoType MTBDR*plus* assay (Hain Lifescience), following the manufacturer's protocol (Barnard et al., 2008).

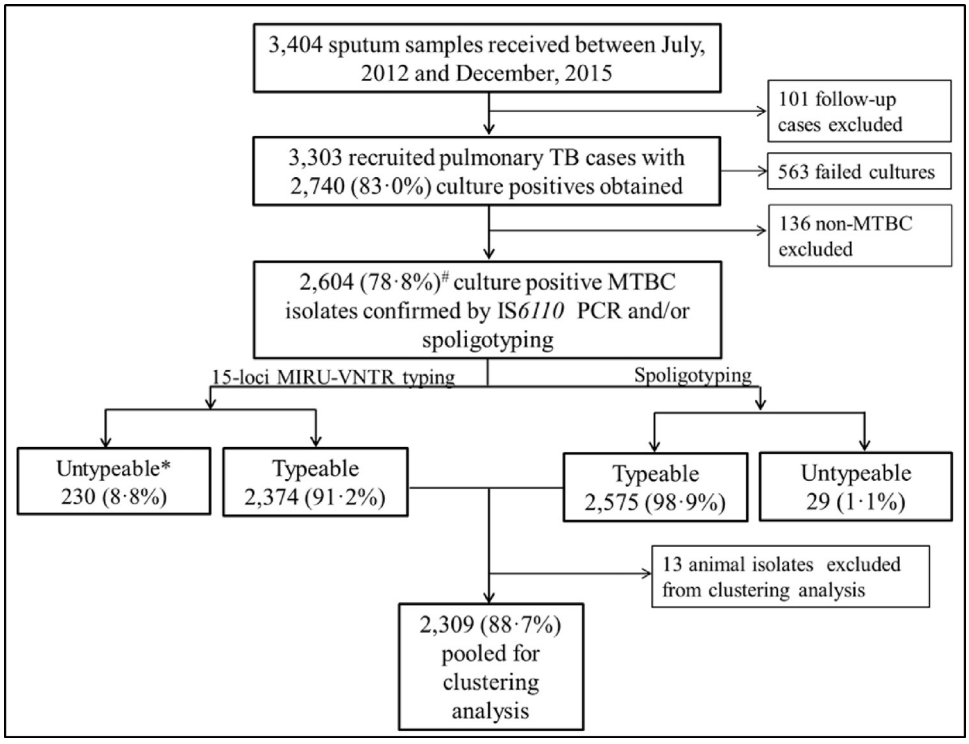


**Figure 1.** Pipeline for recruited participants and culture-positive TB cases included in the clustering analysis.
*Category described as untypeable for MIRU-VNTR includes isolates with ≥2 MIRU loci unamplified ($n$ = 164, 71.3%) and isolates with a double allele at ≥2 MIRU loci ($n$ = 66, 28.7%). These isolates were described as suspected mixed infection or laboratory contamination and hence were excluded from further analysis.
#Frequency was expressed as the total number of *Mycobacterium tuberculosis* complex (MTBC) isolates obtained.