学位論文内容の要旨
Abstract of the dissertation

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Molecular epidemiological study of multidrug-resistant *Mycobacterium tuberculosis* in Lusaka, Zambia
（ザンビア、ルサカ市における多剤耐性結核の分子疫学的研究）

Objective: Zambia is among the 30 high tuberculosis burden countries in the world. Despite increasing reports of multidrug-resistant tuberculosis (MDR-TB) in routine surveillance, information on the transmission of MDR *Mycobacterium tuberculosis* strains is largely unknown. This study elucidated the genetic diversity and transmission of MDR *M. tuberculosis* strains in Lusaka, Zambia.

Methods: Eighty-five MDR *M. tuberculosis* samples collected from 2013 to 2017 at the University Teaching Hospital were used in this study. Drug-resistance associated gene sequencing, spoligotyping, 24-loci mycobacterial interspersed repetitive units-variable number of tandem repeats (MIRU-VNTR) were applied. Additionally, multiplex PCR was used for RD-Rio sub-lineage identification. Further, twelve of the MDR CAS1-Kili isolates clustered by the traditional genotyping methods (24-loci MIRU-VNTR and spoligotyping) were investigated for recent transmission using whole-genome sequencing (WGS) on Illumina MiSeq platform.

Results: The identified clades were LAM (48%), CAS (29%), T (14%), X (6%) and Harlem (2%). Many strains belonged to SIT 21/CAS1-Kili (29%) and SIT 59/LAM11-ZWE (19%). Strains belonging to SITs 21/CAS1-Kili and 20/LAM1 formed the largest clonal complexes. The combined spoligotyping and 24-loci MIRU-VNTR revealed 47 genotypic patterns with a clustering rate of 63%. Among the LAM strains, ninety-five percent belonged to the RD-Rio sub-lineage. Of the 12 CAS1-Kili strains, 92% (11/12) belonged to a cluster as they differed by less than or equal to 12 SNPs. While 50% (6/12) were involved in recent transmission events, as they differed by less than or equal to 5 SNPs. All the 12 strains had KatG Ser 315 Thr (isoniazid resistance), EmbB Met 306 substitutions (ethambutol resistance), and several kinds of rpoB mutations (rifampicin resistance). WGS also revealed compensatory mutations including a

novel deletion in *embA* regulatory region (−35A > del). Several strains shared the same combinations of drug-resistance-associated mutations indicating transmission of MDR strains. The 12 Zambian strains belonged to the same clade as Tanzanian, Malawian, and European strains, although most of those from other countries were pan-drug-susceptible.

Conclusion: The high clustering rate by MIRU-VNTR and spoligotyping suggested that a large proportion of MDR-TB spread was due to recent transmission rather than the independent acquisition of MDR. This spread was attributed to clonal expansion of SIT 21/CAS1-Kili and SIT 20/LAM1 strains. Besides, WGS showed sequential acquisition of drug-resistance associated mutations in addition to several compensatory mutations among the 12 CAS1-Kili strains. Therefore, TB control programs involving genotyping coupled with conventional epidemiological methods were recommended. Further, the study supported complimentary use of WGS to traditional epidemiological methods as it provides an in-depth insight on transmission and drug resistance patterns which can guide targeted control measures to stop the spread of MDR-TB.