



HOKKAIDO UNIVERSITY

Title	Streptomycin and ethambutol resistance associated mutations of multidrug-resistant Mycobacterium tuberculosis clinical isolates in Lusaka, Zambia [an abstract of dissertation and a summary of dissertation review]
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Degree Grantor	北海道大学
Degree Name	博士(感染症学)
Dissertation Number	甲第15047号
Issue Date	2022-03-24
Doc URL	https://hdl.handle.net/2115/86011
Rights(URL)	https://creativecommons.org/licenses/by/4.0/
Type	doctoral thesis
File Information	BWALYA_Precious_abstract.pdf, 論文内容の要旨



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

博士の専攻分野の名称：博士（感染症学）

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学位論文題名
The title of the doctoral dissertation

**Streptomycin and ethambutol resistance associated mutations of
multidrug-resistant *Mycobacterium tuberculosis* clinical isolates in Lusaka,
Zambia**

（ザンビアで分離された多剤耐性結核菌におけるストレプトマイシンおよびエタンブトール耐性関連遺伝子変異の特徴）

In Zambia, multidrug-resistant tuberculosis (MDR-TB) is an emerging threat and has been increasing in recent years. The increasing trend is an urgent public health concern. Previous studies have shown that transmission of drug-resistant *Mycobacterium tuberculosis* (*Mtb*) is a major contributor to the increasing cases of MDR-TB in Zambia. Delayed or inaccurate drug susceptibility test (DST) results lead to ineffective treatment of MDR-TB and patients remaining infectious, thereby spreading resistant strains. Therefore, there is an urgent necessity to adopt a rapid molecular-based tool to improve the diagnosis of MDR-TB and initiate effective treatment to reverse the trend. This study was aimed at evaluating molecular analysis using DNA sequencing for DST of streptomycin (STR) and ethambutol (EMB), drugs comprised in the treatment of MDR-TB in Zambia, and characterize mutations conferring resistance to these drugs. Additionally, an evaluation of the correlation between drug resistance-conferring mutations and *Mtb* genotypes was done.

In chapter I, DNA sequencing of *rpsL*, *rrs*, and *gidB* genes associated STR resistance revealed that mutations in *rpsL* and *rrs* were exclusively found in STR resistant isolates. However, some mutations in *gidB* known to confer low-level STR resistance were mischaracterized as susceptible by phenotype drug susceptibility testing (DST). Thus DNA sequencing would improve resistance STR resistance detection. A correlation between CAS1_Kili genotype

and STR resistance as well as with *rpsL* amino acid substitution K43R was found. K43R is known to be associated with the Beijing genotype, thus the unique association with CAS1_Kili in this study suggests clonal expansion. Conversely, the LAM1 genotype was not associated with STR but, could be considered as a cluster due to harboring the same *rpoB* and *katG* mutations. Possibly, this genotype was introduced to Zambia as an MDR-TB strain after STR was no longer used as a first-line drug and has since continued to spread.

In Chapter II, *embB* gene sequencing and phenotypic DST detected EMB resistance in 75.8% and 46.2% of MDR-TB isolates, respectively. Mutations in *embB* were associated with resistance to at least 3 drugs, suggesting drug resistance amplification in strains with *embB* mutations. This stresses the danger of inaccurate EMB susceptibility results by phenotype DST. CAS1_Kili was associated with *embB* mutations particularly, amino acid substitution M306I. The study also revealed a unique predilection of CAS1_Kili and Lineage 2 to acquire amino acids M306I and M306V, respectively, stressing the possible influence strain genetic background has on drug resistance pathways.

This study effectively demonstrated the adequacy of DNA sequencing DST of STR and EMB in Zambia. This test method has increased scope, is rapid, and is sensitive compared to phenotypic testing. In addition, mutation analysis enabled the detection of MDR-TB transmission clusters. Thus, the adoption of DNA sequencing for DST would improve MDR-TB management and halt the upward trend of MDR-TB in Zambia. The association of CAS1_Kili with drug resistance in this study makes it a genotype of concern in Zambia and should be closely monitored.