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

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学位論文題名
The title of the doctoral dissertation

Molecular analysis of drug resistance associating gene mutations in *Mycobacterium tuberculosis* clinical isolates from Nepal

(ネパールで分離された結核菌の薬剤耐性関連遺伝子変異の解析)

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (MTB), is a major cause of public health problems, and one of the top ten causes of death worldwide. With an increasing number of drug-resistant TB (DR-TB) each year, effective treatment of TB possesses challenges. The time consuming conventional proportional method using LJ medium is the only available DST in Nepal. Although multi DR-TB (MDR-TB) is one of the emerging threats in Nepal, limited studies had been reported to determine the prevalence of drug resistance-conferring mutations among those isolates.

In chapter I, I have described the characterization of the molecular mechanism of streptomycin (STR) resistance MTB isolates from Nepal. Mutation in *rpsL* (encoding ribosomal protein S12), *rrs* (encoding 16S ribosomal RNA), and *gidB* (encoding 7-methylguanosine methyltransferase) are associated with resistance to STR, which is used for the treatment of MDR-TB in Nepal. Mutations in *rpsL* were harbored by 65.9% of isolates, in which the most common mutation in *rpsL* is caused by K43R (58.8%) and was significantly associated with the Beijing genotype ($P<0.001$). About 13.2% of isolates harbored mutations in two highly mutable regions of *rrs*, the 530 loop and the 912 region. About 13.2% of *gidB* mutants do not show any mutation in *rpsL* and *rrs*, which might suggest the role of *gidB* mutations in STR resistance in MTB. Also, 5.6% of isolates do not show any mutations in the three genes examined, suggesting the involvement of other mechanisms in STR resistance in MTB.

Without the proper information of pyrazinamide (PZA) susceptibility of MTB, PZA is inappropriately recommended for the treatment of both susceptible and MDR-TB in Nepal. In chapter II, I attempted the first insight to collect information regarding PZA

susceptibility in MTB isolates from Nepal by analyzing mutations in *pncA*. Sequence analysis of *pncA* and its upstream regulatory region (URR) was performed to assess PZA resistance. The sequencing results revealed that 125 (59.2%) isolates harbored alterations in *pncA* and its URR. I detected 57 different mutation types (46 reported and 11 novels) that were scattered throughout the whole length of the *pncA*. I identified 87 (41.2%) isolates harbored mutations in *pncA* causing PZA resistance in MTB. There was a significant association of *pncA* alterations among MDR/pre-extensively drug-resistant (Pre-XDR) TB than among non-MDR-TB ($p<0.005$). The rate of *pncA* mutation was high in MDR-TB/Pre-XDR-TB, and most of the *pncA* mutations resulted in PZA resistance in MTB. The increasing number of PZA resistance among DR-TB in Nepal highlights the importance of PZA susceptibility testing for DR-TB treatment. Considering the long turnaround time of phenotypic DST in Nepal, we recommend the more rapid method of molecular *pncA* sequencing for detection of PZA susceptibility.

The findings from this study could provide the knowledge on molecular drug resistance mechanism of anti-TB drugs of STR and PZA. The information of frequency and patterns of drug resistance-associated mutations can be implemented for the establishment of rapid and accessible molecular DST tools, in which TB can be treated with appropriate drugs and can improve control strategies for DR-TB in Nepal.