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学位論文全文の要約

Summery of the dissertation

博士の専攻分野の名称:博士(獣医学) 氏名: Dipti Shrestha

Molecular analysis of drug resistance associating gene mutations in *Mycobacterium tuberculosis* clinical isolates in 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析)

With an increasing multidrug-resistant TB in the world, Nepal is not an exception. Even though TB is ranked as the top seventh disease to cause death in Nepal, limited studies have been conducted to determine the phenotypic and genotypic drug-resistant TB in Nepal. For the effective treatment and control of DR-TB in Nepal, a rapid diagnostic tool for DST is required. For the development of a rapid DST tool, information regarding the frequency and patterns of drug resistance associating gene mutations among DR-TB isolates is necessary.

In chapter I of this thesis, 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. 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. In addition, 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. Our findings of mutations in *rpsL*, *rrs*, and *gidB* satisfactorily predict the STR-resistant MTB in Nepal and can be implemented for the establishment of molecular STR-susceptibility testing, in which tuberculosis can be treated with appropriate drugs and can improve control strategies for DR-TB.

In chapter II, I aimed to collect information regarding pyrazinamide susceptibility in MTB isolates from Nepal by analyzing mutations in *pncA*. I investigated the frequency and patterns of *pncA* mutation among MTB isolates. I analyzed the *pncA* mutations by comparing with previously reported PZA resistance associating *pncA* mutations and in-vivo/in-vitro study. In addition, I described the *pncA* mutations by analyzing the single nucleotide substitution of *pncA* with the webserver predictive tool (SUSPECT-PZA). A total of 211 MTB isolates collected from August 2008 to February 2011 were included in this study. Sequence analysis of *pncA* and its upstream regulatory region was performed to assess PZA resistance. First-line drug susceptibility testing, spoligotyping, and sequence analysis of *rpoB*, *katG*, *inhA* regulatory region, *gyrA*, *gyrB*, and *rrs* were performed to assess their association with *pncA* mutations. The sequencing results revealed that 125 (59.2%) isolates harbored alterations in *pncA* and its upstream regulatory region. We detected 57 different mutation types (46 reported and 11 novels) that were scattered throughout the whole length of the *pncA* gene. 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 feasible method of molecular *pncA* sequencing for the detection of PZA susceptibility.

The findings from this study can provide the elaborated knowledge on the genotypic drug resistance mechanism of STR and PZA. This study focused on the routine DST before the implementation of drugs for DR-TB treatment in Nepal. Moreover, this study was focused on frequency and patterns in drug-resistance-associating gene mutations which can help in the development of easy, rapid, and accessible DST tools. The diagnostic tools could contribute to policymaking on proper management of DR-TB treatment through early diagnosis and appropriate DR-TB treatment in Nepal.