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 characterization of *Mycobacterium avium* clinical isolates from Japan and development of diagnostic tools

(日本の臨床分離トリ型結核菌株の遺伝子解析と診断法開発)

Mycobacterium avium (*M.avium*) is the leading cause of nontuberculous mycobacterial lung disease worldwide. Early detection and treatment are paramount for the timely control of *M. avium* infections.However, the lack of affordable rapid diagnostic tools and emergence of drug resistance among other factors has made the management of *M. avium* infections very challenging. And the emergence of macrolide resistance has resulted in the use of fluoroquinolones (FQ) to treat drug resistant *M. avium* infections. Recently, FQ resistance has been increasing and in *Mycobacterium tuberculosis*, resistance is largely attributed to mutations in FQ resistance associating genes, *gyrA* and *gyrB*. However, information on FQ resistance mechanisms in *M. avium* is limited.

In Chapter I, a rapid detection LAMP-based assay was developed targeting a widely used species-specific marker IS*1245*. The applicability of this assay was further assessed using human (n = 137) and pig (n = 91) *M. avium* isolates from Japan. The LAMP assay had a detection limit of 6 fg (equivalent to 1 genome copy) of *M. avium* DNA per reaction within 30 minutes. All 91 (100%) *M. avium* isolates from pigs were detected positive while all other tested bacterial species were negative. Interestingly, among the 137 clinical *M. avium* isolates, 41 (30%) were undetectable with this LAMP assay as they lacked the IS*1245*, the absence of which was revealed by PCR and whole-genome sequencing. These findings highlighted genotypic differences in *M. avium* strains from humans and pigs in Japan and how this diversity can influence the applicability of a detection tool across different geographic areas and hosts.Hence, it is critical to understand the local genetic make-up of a pathogen population before adopting internationally set standards. This fast and cost-friendly method will aid in the rapid diagnosis of *M. avium* especially in the Euro Americas which have reported high IS*1245* carriage rates and result in timely treatment.

In Chapter II, the mechanisms of resistance to levofloxacin (LVX), a FQ, were investigated through minimum inhibitory concentration (MIC) determination and sequencing of *gyrA*, *gyrB*, *mfpA*, and *mfpB* in a total of 88 *M. avium* isolates from Japan. Among the isolates, 21.6 % (19/88)were susceptible to LVX, whereas 78.4 % (69/88), were resistant. Since only four of the resistant isolates

(4/88, 4.5 %) harbored resistance-conferring mutations (D94Y or D94G) within the *QRDR* of *gyrA*, the role of *mfpA* in LVX resistance was explored.Two *mfpA* genotypes were observed by whole-genome sequencing (WGS) and to simplify the analysis, a multiplex PCR was developed to rapidly detect these genotypes (C170deleted *mfpA* and Intact/Non-C170deleted *mfpA*).From the total 88 isolates analyzed in this study, 58 had the intact *mfpA* genotype while 30 had C170deleted *mfpA* type. Among the 58 isolates with intact *mfpA*, 53/55 (96.4%) were resistant to LVX.MIC determination revealed a significant association ($p < 0.001$) between isolates with intact-*mfpA* genotype and decreased LVX susceptibility in comparison with isolates bearing the C170deleted *mfpA*. This observation suggested that *mfpA* plays some role in FQ resistance in *M.avium* and therefore warrants more attention.

Overall, the findings of my studies are intended to contribute to the control of *M.avium* infections.