Chapter 1

Introduction

1.1 Background

Mycobacterium abscessus complex (MABSC) is a group of nontuberculous mycobacteria (NTM) that have emerged as significant opportunistic pathogens, globally. MABSC causes a wide range of infections from skin and soft tissue infections to chronic pulmonary diseases, particularly in immunocompromised individuals and those with underlying lung conditions such as cystic fibrosis (Lopeman et al., 2019). The increasing prevalence of MABSC infections, in combination with their intrinsic resistance to an array of antimicrobial agents, poses a critical challenge to clinical management and public health (Johansen et al., 2020).

MABSC is subdivided into three subspecies including *M. abscessus* subsp. *abscessus* (MAB), *M. abscessus* subsp. *massiliense* (MMA), and *M. abscessus* subsp. *bolletii* (MBO). These subspecies display distinct phenotypic and genotypic profiles, particularly antimicrobial susceptibility (Sukmongkolchai et al., 2023). A key difference is an inducible clarithromycin resistance mediated by the erythromycin ribosomal methylase gene, *erm(41)*. *Mycobacterium abscessus* shows to have *erm(41)* gene, which enables inducible resistance (Nash et al., 2009), whereas MMA possesses a truncated version of the gene, rendering it nonfunctional (Kim et al., 2010). In addition, acquired resistance to macrolides, such as clarithromycin, can arise from point mutations in the *rrl* gene encoding 23S rRNA, particularly at positions 2058 and 2059 within the peptidyl transferase region (Realegeno et al., 2021). Similarly, resistance to amikacin is primarily associated with mutations in the *rrs* gene encoding 16S rRNA, most notably at position 1408 (Nessar et al., 2011).

MABSC exhibits two distinct colony morphotypes, which are smooth (S) and rough (R) (Rüger et al., 2013). This morphological difference is primarily attributed to the biosynthesis of glycopeptidolipids (GPLs), which are crucial molecules for colony morphology and pathogenicity (Gutiérrez et al., 2018).

The production and transport of GPLs are regulated by genes within the GPL biosynthetic locus, such as *mps1*, *mps2*, *mmpL4b*, and *mmps4*. Isolates with a smooth colony morphotype actively synthesize and display GPLs on their cell wall, which is associated with a less invasive phenotype (Howard et al., 2006). In contrast, mutations or disruptions within the *gpl* locus can result in a reduction or complete loss of GPL production, leading to a rough morphotype. This rough variant lacks surface GPLs and is typically associated with enhanced virulence and more aggressive disease progression (Hedin et al., 2023).

Another key factor contributing to the persistence and treatment failure of MABSC infections is its ability to form biofilms (Fennelly et al., 2016; Helou et al., 2013; Qvist et al., 2015). Biofilms are structured bacterial communities encased in a self-produced extracellular polymeric matrix that acts as a barrier to evade host immune responses, and limit antibiotic penetration and efficacy (De Santana Lira et al., 2025). The formation of these complexes enables MABSC to survive under hostile environmental conditions and persist within the host, contributing to chronic or relapsing infections.

Despite the growing global burden of *Mycobacterium abscessus* complex (MABSC) infections, comprehensive local epidemiological data, particularly integrating phenotypic characteristics with genomic insights, remain limited. Therefore, this study aimed to characterize a collection of clinical MABSC isolates obtained from patients at KCMH, Bangkok, Thailand. Specifically, we investigated subspecies distribution, colony morphotypes, antimicrobial susceptibility profiles, and biofilm-forming capacity in order to provide a deeper knowledge of the clinical and microbiological features of MABSC and to support improved diagnostic and therapeutic approaches.

1.2 Objective

- To determine the subspecies distribution and colony morphotypes of MABSC clinical isolates
- To assess their antimicrobial susceptibility profiles, specifically amikacin and clarithromycin
- To evaluate their capacity for biofilm formation
- To elucidate the genotype underlying observed phenotypes, including antimicrobial resistance (*erm(41)*, *rrl*, *rrs*) and colony morphotype (*gpl* locus genes including *mps1*, *mps2*, *mmpS4*, and *mmpL4b*)

1.3 Hypothesis

1. The majority of clinical *Mycobacterium abscessus* complex (MABSC) isolates will exhibit resistance to both amikacin and clarithromycin.
2. Isolates with a rough colony morphotype will demonstrate reduced or absent biofilm formation due to the loss or disruption of glycopeptidolipid (GPL) synthesis.
3. Genetic analysis will reveal consistent mutations in known antimicrobial resistance genes (*erm(41)*, *rrl*, and *rrs*) corresponding to the observed phenotypic resistance profiles.