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KatG as Counterselection Marker for Nontuberculous Mycobacteria

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In a recent issue of *Antimicrobial Agents and Chemotherapy*, Reingewertz et al. (1) report on sensitization of slow-growing, nontuberculous mycobacteria (NTM) to the anti-tubercular drug isoniazid (INH) upon expression of *Mycobacterium bovis* KatG.

KatG functions as a catalase-peroxidase (2, 3) and activates INH, which then inhibits InhA, an enzyme involved in mycolic acid synthesis (4). However, a surge in INH-resistant *Mycobacterium tuberculosis* clinical isolates is jeopardizing the role of INH as a first-line drug (5). In general, resistance to INH can be acquired by mutations in *katG* or, less frequently, in the promoter region of *inhA* (6, 7). In the first case, KatG no longer activates INH, while in the second case, a higher tolerance to the drug is conferred by increased InhA expression (8).

The aim of the aforementioned study was to elucidate the differences between KatG-dependent INH activation in mycobacteria and its effect on INH susceptibility, focusing on the opportunistic pathogens *Mycobacterium avium* subsp. *paratuberculosis* and *Mycobacterium marinum* (both NTM and naturally refractory to INH).

NTM are mycobacteria not belonging to the *M. tuberculosis* complex and encompass both slowly and rapidly growing mycobacterial species (SGM and RGM) (9). As shown by our colleagues and other groups (1, 10–12), NTM usually show an innate decreased susceptibility toward INH. Within NTM, RGM have significantly higher INH MICs than SGM (11–13). The reason for this increased resistance is likely the result of several factors, including a failure to activate the prodrug, target-level mutations, differences in the C-terminal domain of KatG (3), the reduction of intracellular concentration (by means of efflux pumps or decreased permeability) (14), and/or the possible nonessentiality of the mycolic acid synthesis pathway in NTM (12). However, essentiality has been proven by identifying pyridomycin as a specific inhibitor of InhA preventing growth of both *M. tuberculosis* (MIC = 0.39 mg/liter) and NTM (*M. marinum* MIC = 3.13 mg/liter) (15).

In the context of NTM, no other mycobacteria have proven to be as resilient as the emerging opportunistic pathogens from the *Mycobacterium abscessus* complex (16). As members of the RGM (9), their intrinsic antibiotic resistance through drug- and target-modifying enzymes (17) has rendered *M. abscessus* complex infections extremely challenging to treat. Lengthy regimens on multiple drugs with severe side effects are the norm (11, 13). KatG phylogeny pinpointed *M. abscessus* complex as being closer to *M. tuberculosis* than *rpoB*-based phylogeny did (1). Alignment of $KatG^{Mabs}$ with $KatG^{Mtb}$ shows that the most common INH resistance-conferring clinical *M. tuberculosis* mutations (18) are absent in *M. abscessus* ATCC 19977 and sequence identity is high (approximately 72%).

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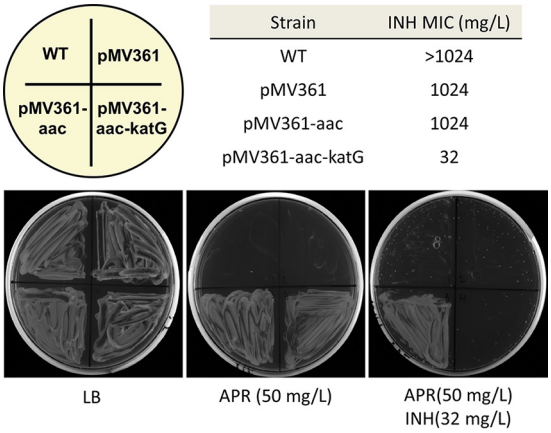

Strain	INH MIC (mg/L)
WT	>1024
pMV361	1024
pMV361-aac	1024
pMV361-aac-katG	32

FIG 1 INH susceptibility of *M. abscessus* complex strains upon KatG^{Mtb} expression. MICs (day 7) for the strains used to generate *M. abscessus* complex pMV316-aac-katG (unpublished data) are shown. MICs were measured by broth microdilution assay (25). Luria-Bertani (LB) agar with APR and with APR-INH shows selective growth of *M. abscessus* complex.

Our research group has taken advantage of the resistance of *M. abscessus* complex to INH in a manner similar to that used by Reingewertz and coworkers. In a proof-of-concept study, we heterologously expressed KatG^{Mtb} in *M. abscessus* complex from a pMV361 (19) *attB*-integrative vector containing an apramycin (APR) resistance cassette for selection (*aac*). This allowed us to develop new tools for the genetic manipulation of *M. abscessus* complex and to use INH as an effective counterselection marker for allelic replacements (20–24), even if the MIC was well above achievable therapeutical concentrations (MIC = 32 mg/liter) (Fig. 1). Our observations are in strong agreement with the work from Reingewertz et al., showing a drop in MIC of approximately 30-fold, and are proof that INH susceptibility can be successfully exploited. However, the comparatively high MIC of recombinant *M. abscessus* complex pMV361-aac-katG indicates that besides poor KatG-dependent INH activation, other factors contribute to the high level INH resistance of *M. abscessus*.

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