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Letter to the editor

The *in vitro* mechanisms of isoniazid and ethionamide resistance poorly reflect those *in vivo* in *Mycobacterium tuberculosis*

Florence BROSSIER^{a,b,c,*}, Emmanuelle CAMBAU^{a,d,e}, Emilie TESSIER^a, Vincent JARLIER^{a,b,c}, Wladimir SOUGAKOFF^{a,b,c}

^a National Reference Center for Mycobacteria and Antimycobacterial Resistance, Paris, France.

^b AP-HP, Hôpital Pitié-Salpêtrière, Laboratory of Bacteriology, Paris, France.

^c Sorbonne Universités, UPMC Univ Paris 06, INSERM, UMRS1135, CIMI-Paris, team 13, Paris, France.

^d AP-HP, Hôpital Lariboisière, Laboratory of Bacteriology, Paris, France.

^e Univ Paris Diderot, IAME UMR1137, Sorbonne Paris Cité, Paris, France.

* Correspondence to Florence Brossier, Laboratoire de Bactériologie-Hygiène, Faculté de Médecine, Pitié-Salpêtrière, 91 boulevard de l'Hôpital, F-75634 Paris Cedex 13, France.
Phone: (+33) 1 40 77 97 46. Fax: (+33) 1 45 82 75 77. E-mail: florence.brossier@aphp.fr

To be active against *Mycobacterium tuberculosis*, isoniazid (INH) and ethionamide (ETH) need to be activated by the catalase/oxidase KatG for INH and by the mono-oxygenase EthA (regulated by EthR) for ETH [1]. ETH and INH both target the enzyme InhA involved in the cell wall biosynthesis [2]. Clinical isolates resistant (R) to INH and ETH display a wide range of mutations altering KatG (mutation S315T in 70% of INH-R isolates), EthA (in 50% of ETH-R isolates) and, InhA and the *inhA* promoter (in 25% of INH-R and 65% of ETH-R isolates) [1, 3].

Here we selected INH-R and ETH-R *in vitro* mutants in order to investigate, in an isogenic context, the effects of the mutations observed in clinical isolates. Mutants were selected on Middlebrook 7H11 agar containing INH or ETH. The genes *ethA*, *ethR*, *katG*, *inhA* and the *inhA* promoter were sequenced for 115 *M. tuberculosis in vitro* mutants as previously described [3].

The mutants selected on INH displayed alterations in *katG* only (46/69), with the following mutations: missense (12/69), nonsense (14/69), insertion (2/69), partial and complete deletion of *katG* (13/69 and 5/69, respectively) (Table 1). The mutants selected on ETH showed alteration in *ethA* (11/46) and the *ethA-ethR* intergenic region (1/46), with the following mutations in *ethA*: missense (5/46), nonsense (4/46) and deletion (2/46) (Table 1).

The distribution of the mutations selected *in vitro* does not reflect the mechanisms of INH- and ETH-R usually observed in clinical isolates. The most common mutations found in the INH-R clinical isolates (KatG S315T and *inhA* promoter c-15t) were not found in our *in vitro* INH-R mutants which, by contrast, preferentially showed deletions in *katG* (46%), a genotype that is rarely observed in clinical isolates [1]. This observation is consistent with Bergval et al for INH [4]. However, we observed more missense mutations (12/69 vs 2/120) and the mutations we obtained were selected with the lowest concentrations of INH (0.2 and 0.4

mg/l), compared to Bergval et al [4] who performed the selection of resistant mutants at concentrations of 0.4, 1.0 and 20 mg/l.

KatG allows bacteria to resist killing by the phagocyte oxidative burst happening when *M. tuberculosis* are in contact with the host's immune system [1, 5]. The mutation KatG S315T predominantly found in clinical INH-R strains represents the best compromise between the conservation of catalase-peroxidase activities and a drastic reduction in the INH activation [1]. The high rate of deletion in KatG observed in *in vitro* mutants, which are resistant to isoniazid but susceptible to oxidative burst, could be favored by the *in vitro* culture conditions where there is no significant oxidative stress.

Similarly, the c-15t mutation in the *inhA* promoter, commonly found in clinical isolates co-resistant to INH and ETH, was not observed in our ETH-R mutants which displayed mutations only in EthA and the *ethA-ethR* intergenic region. Finally, for 33% and 74% of the INH-R and ETH-R mutants obtained *in vitro*, respectively, no mutation was found in *ethA*, *ethR*, *katG*, *inhA* and the *inhA* promoter.

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88 Table 1. KatG and EthA mutations found in strains of *Mycobacterium tuberculosis* selected in
89 *vitro* by isoniazid or by ethionamide

Resistance phenotype (no. of isolates)	Gene	Type of mutations (no. of isolates)	Mutation ^a (no. of isolates indicated if different of 1)
Isoniazid (n=69)	<i>katG</i>	missense (n=12)	D137Y, F183V, <u>P232L</u> (n=2), <u>T275I</u> , <u>S315C</u> , W348S, T394M, <u>R484H</u> (n=3), G494D
		nonsense (n=14)	Q50*, <u>W91*</u> (n=2), W135*, K152*, <u>W328*</u> (n=2), W351*, W398*, Q500*, Y638*, Q679*
		insertion (n=2)	ins g 1110, ins a 1604
		deletion (n=18)	
		° partial (n=13)	del c 351, del g 367, del aagaac 469-475, del t 548, del catc 1391-1394 (n=8), del a 1668
		° total (n=5)	
		wild-type (n=23)	
Ethionamide (n=46)	<i>inhA</i> or its promoter	wild-type (n=69)	
	<i>ethA</i>	missense (n=5)	T44N , G52E, A252D, A341V (n=2)
		nonsense (n=4)	Q360* (n=2), W456* (n=2)
		deletion (n=2)	del g 88, del a 256
		wild-type (n=35)	
	<i>ethA-ethR</i> intergenic region	single base a-68g (n=1)	
		wild-type (n=45)	
	<i>ethR</i>	wild-type (n=46)	
	<i>inhA</i> or its promoter	wild-type (n=46)	

90 ^a The mutated codons previously reported in the literature in clinical strains are indicated in
91 bold when the corresponding amino acid is conserved, or are underlined when the encoded
92 amino acid is modified compared to our study [1].