

# Impact of heteroresistance on treatment outcomes of people with drug-resistant TB

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**Table S1. Mutation pattern in each of 624 MTB isolates**

<b>Mutation</b>	<b>Resistant n (%)</b>	<b>Susceptible n (%)</b>
<b>Isoniazid (615 resistant MTB isolates with valid data)</b>		
<b>katGS315T</b>	360 (58.5)	
<b>inhAc-15t</b>	159 (25.9)	
<b>inhAc-15t, katGS315T</b>	37 (6.0)	
<b>katGS315N</b>	16 (2.6)	
<b>inhA KS315N</b>	1 (0.2)	
<b>No mutation</b>	42 (6.8)	
<b>Rifampicin (624 resistant MTB isolates), All mutations in the <i>rpoB</i> gene</b>		
<b>S531L</b>	375 (60.1)	
<b>S531L H526Y</b>	3 (0.5)	
<b>S531L S531F</b>	2 (0.3)	
<b>S531L H526L</b>	2 (0.3)	
<b>S531L H526D</b>	2 (0.3)	
<b>S531L D516V</b>	2 (0.3)	
<b>S531L S531W</b>	1 (0.2)	
<b>S531L S522Q</b>	1 (0.2)	
<b>S531L S522L</b>	1 (0.2)	
<b>S531L L533P</b>	1 (0.2)	
<b>S531L H526Y D516Y L533P</b>	1 (0.2)	
<b>S531L H526R L533P</b>	1 (0.2)	
<b>H526Y</b>	58 (9.3)	
<b>H526Y H526C H526R</b>	1 (0.2)	
<b>H526Y H526R</b>	1 (0.2)	
<b>H526R</b>	23 (3.7)	
<b>H526R L533P</b>	1 (0.2)	
<b>H526D</b>	23 (3.7)	
<b>H526D Q513K</b>	4 (0.6)	
<b>S531W</b>	19 (3.0)	
<b>D516V</b>	19 (3.0)	
<b>H526C</b>	14 (2.2)	
<b>L533P</b>	11 (1.8)	
<b>H526L</b>	8 (1.3)	
<b>H526L D516V</b>	1 (0.2)	
<b>D516Y</b>	8 (1.3)	
<b>S522L</b>	8 (1.3)	
<b>Q513P</b>	3 (0.5)	

<b>D516F</b>	3 (0.5)	
<b>Q513L</b>	2 (0.3)	
<b>S531F</b>	1 (0.2)	
<b>S531F D516V</b>	1 (0.2)	
<b>Q513K</b>	1 (0.2)	
<b>No mutation</b>	22 (3.5)	
<b>Fluoroquinolones</b> (105 resistant, 502 susceptible MTB isolates with valid data). All mutations in the <i>gyrA</i> gene.		
<b>D94G</b>	26 (24.8)	1 (0.2)
<b>D94G A90V</b>	3 (2.9)	
<b>D94G A90V D94N</b>	3 (2.9)	
<b>D94G A90V D94A</b>	2 (1.9)	
<b>D94G A90V D94H</b>	2 (1.9)	
<b>D94G D94A</b>	2 (1.9)	
<b>D94G D94H D94Y</b>	1 (1.0)	
<b>D94G D94N</b>	1 (1.0)	
<b>D94G A90V S91P D94A D94H</b>		
<b>D94N D94Y</b>	1 (1.0)	
<b>D94G A90V S91P</b>	1 (1.0)	
<b>D94G G88C A90V S91P</b>	1 (1.0)	
<b>D94G S91P</b>	1 (1.0)	
<b>A90V</b>	22 (21.0)	2 (0.4)
<b>A90V S91P</b>	2 (1.9)	
<b>A90V D94A</b>	1 (1.0)	
<b>D94A</b>	7 (6.7)	
<b>D94N</b>	7 (6.7)	
<b>D94Y</b>	5 (4.8)	
<b>D94H</b>	2 (1.9)	
<b>S91P</b>	1 (1.0)	
<b>No mutation</b>	14 (13.3)	499 (99.4)
<b>Second-line Injectables</b> (134 resistant, 490 susceptible MTB isolates with valid data). All mutations in the <i>rrs</i> gene.		
<b>1401G</b>	106 (79.1)	5 (1.0)
<b>1484T</b>	0 (0.0)	1 (0.2)
<b>No mutation</b>	28 (20.9)	484 (98.8)
<b>Streptomycin</b> (293 resistant, 301 susceptible MTB isolates with valid data). All mutations in the <i>rrs</i> gene.		
<b>A514C</b>	123 (42.1)	7 (2.3)
<b>C517T</b>	9 (3.1)	2 (0.7)
<b>No mutation</b>	161 (54.9)	292 (97.0)

**Table S2. Frequency of mutations per drug class**

<b>Mutation</b>	<b>Resistant n (%)</b>	<b>Susceptible n (%)</b>
<b>Isoniazid</b> (611 mutations among INH resistant MTB)		
<b>katGS315T</b>	397 (65.0)	
<b>inhAc-15t</b>	197 (32.2)	
<b>katGS315N</b>	17 (2.8)	
<b>Rifampicin</b> (632 mutations among RIF resistant MTB, 26 isolates had more than one mutation)		
<b>S531L</b>	392 (62.0)	
<b>H526Y</b>	64 (10.1)	
<b>H526D</b>	29 (4.6)	
<b>H526R</b>	27 (4.3)	
<b>D516V</b>	23 (3.6)	
<b>S531W</b>	20 (3.2)	
<b>H526C</b>	15 (2.4)	
<b>L533P</b>	15 (2.4)	
<b>H526L</b>	11 (1.7)	
<b>D516Y</b>	9 (1.4)	
<b>S522L</b>	9 (1.4)	
<b>Q513K</b>	5 (0.8)	
<b>S531F</b>	4 (0.6)	
<b>D516F</b>	3 (0.5)	
<b>Q513P</b>	3 (0.5)	
<b>Q513L</b>	2 (0.3)	
<b>S522Q</b>	1 (0.2)	
<b>Fluoroquinolones</b> (128 mutations among FQ resistant and 3 among FQ susceptible MTB)		
<b>D94G</b>	44 (34.4)	1 (33.3)
<b>A90V</b>	38 (29.7)	2 (66.6)
<b>D94A</b>	13 (10.2)	
<b>D94N</b>	12 (9.4)	
<b>D94Y</b>	7 (5.5)	
<b>S91P</b>	7 (5.5)	
<b>D94H</b>	6 (4.7)	
<b>G88C</b>	1 (0.8)	
<b>Second-line Injectables</b> (106 mutations among SLI resistant and 6 among SLI susceptible MTB)		
<b>1401G</b>	106 (100.0)	5 (83.3)
<b>1484T</b>	0 (0.0)	1 (16.7)

**Streptomycin** (132 mutations among Streptomycin resistant and 9 among Streptomycin susceptible MTB)

<b>A514C</b>	123 (93.2)	7 (77.8)
<b>C517T</b>	9 (6.8)	2 (22.2)

MTB: Mycobacterium tuberculosis

**Table S3. Clinical and demographic characteristics of 379 patients treated for MDR TB in the Philippines, 2013-2016**

	Total, N (%) N=379	Heteroresistance, n (%) n = 13	No heteroresistance, n (%) n = 366	p-value*
<b>Clinical and demographic characteristics</b>				
Age, median (IQR)	43 (33-52)	52 (42-56)	43 (33-52)	0.06
Female	121 (31.9)	5 (38.5)	116 (31.7)	0.61
Body mass index				
Normal	129 (34.0)	7 (53.9)	122 (33.3)	0.48
Underweight	200 (52.7)	5 (38.5)	195 (53.3)	
Overweight	43 (11.4)	1 (7.7)	42 (11.5)	
Obese	7 (1.9)	0 (0.0)	7 (1.9)	
National Capital Region	112 (29.5)	3 (23.1)	109 (29.8)	0.60
Baseline CXR				
Cavitary	141 (37.2)	7 (53.9)	115 (31.4)	0.23
Non-cavitary	122 (32.2)	3 (23.1)	138 (37.7)	
Unknown	116 (30.6)	3 (23.1)	113 (30.9)	
Smear grade <sup>^</sup>				
Negative or scanty	94 (24.8)	2 (15.4)	92 (25.1)	0.87
(1+)	108 (28.5)	4 (30.8)	104 (28.4)	
(2+)	84 (22.2)	3 (23.1)	81 (22.1)	
(3+)	93 (24.5)	4 (30.8)	89 (24.3)	
Previous TB treatment	365 (96.3)	11 (84.6)	354 (96.7)	0.02
<b>Heteroresistance</b>				
Macro (>10%)	6 (1.6)	6 (46.2)	-	
Micro (≤10%)	2 (0.5)	2 (15.4)	-	
Rare	5 (1.3)	5 (38.5)	-	
Heteroresistance to:				
Fluoroquinolones	2 (0.5)	2 (15.4)	-	
Second-line injectables	1 (0.3)	1 (7.7)	-	
Isoniazid	2 (0.5)	2 (15.4)	-	
Rifampicin	7 (1.9)	7 (53.9)	-	
Streptomycin	2 (0.5)	2 (15.4)	-	
>1 resistant population	16 (4.2)	2 (15.4)	14 (3.8)	0.04

MDR: Multi-drug resistant, TB: tuberculosis, IQR: interquartile range, CXR: Chest x-ray

\*p-value for the association of clinical factor with heteroresistance, using chi-squared test for categorical variables and the Wilcoxon rank-sum to compare median age

<sup>^</sup>Highest pre-treatment smear grade

**Table S4. Clinical and demographic characteristics of 140 patients treated for MDR plus SLIR TB in the Philippines, 2013-2016**

	<b>Total, n (%) N = 140</b>	<b>Heteroresistance, n (%) n = 8</b>	<b>No heteroresistance, n (%) n = 132</b>	<b>p-value*</b>
<b>Clinical and demographic characteristics</b>				
Age, median (IQR)	34 (26-44.5)	34.5 (27-45.5)	34 (26-44.5)	0.94
Female	32 (22.9)	0 (0.0)	32 (24.2)	0.11
Body mass index				
Normal	52 (37.1)	4 (50.0)	48 (36.4)	0.78
Underweight	77 (55.0)	4 (50.0)	73 (55.3)	
Overweight	10 (7.1)	0 (0.0)	10 (7.6)	
Obese	1 (0.7)	0 (0.0)	1 (0.8)	
National Capital Region	102 (72.9)	7 (87.5)	95 (72.0)	0.34
Baseline CXR				
Cavitary	18 (12.9)	0 (0.0)	18 (13.6)	0.27
Non-cavitary	36 (25.7)	1 (12.5)	35 (26.5)	
Unknown	86 (61.4)	7 (87.5)	79 (59.9)	
Smear grade <sup>^</sup>				
Negative or scanty	54 (38.6)	3 (37.5)	51 (38.6)	0.37
(1+)	33 (23.6)	4 (50.0)	29 (22.0)	
(2+)	25 (17.9)	0 (0.0)	25 (18.9)	
(3+)	27 (19.3)	1 (12.5)	26 (19.7)	
Missing	1 (0.7)	0 (0.0)	1 (0.8)	
Previous TB treatment	117 (83.6)	4 (50.0)	113 (85.6)	0.008
<b>Heteroresistance</b>				
Macro (>10%)	5 (3.6)	5 (62.5)	-	
Micro (≤10%)	1 (0.7)	1 (12.5)	-	
Rare	2 (1.4)	2 (25.0)	-	
Heteroresistance to:				
Fluoroquinolones	0 (0.0)	0 (0.0)	-	
Second-line injectables	6 (4.3)	6 (75.0)	-	
Isoniazid	4 (2.9)	4 (50.0)	-	
Rifampicin	6 (4.3)	6 (75.0)	-	
Streptomycin	4 (2.9)	4 (50.0)	-	
>1 resistant population	5 (3.6)	2 (25.0)	3 (2.3)	0.001

SLIR: Second line injectables resistant, TB: Tuberculosis, CXR: Chest x-ray, IQR: interquartile range

\*p-value for the association of clinical factor with heteroresistance, using chi-squared test for categorical variables and the Wilcoxon rank-sum to compare median age

<sup>^</sup>Highest pre-treatment smear grade

**Table S5. Clinical and demographic characteristics of 105 patients treated for Pre-XDR TB in the Philippines, 2013-2016**

	<b>Total, n (%) N = 105</b>	<b>Heteroresistance, n (%) n = 31</b>	<b>No heteroresistance, n (%) n = 74</b>	<b>p-value*</b>
<b>Clinical and demographic characteristics</b>				
Age, median (IQR)	39 (28-54)	49 (34-58)	35 (24-54)	0.01
Female	36 (34.3)	11 (35.5)	25 (33.8)	0.87
Body mass index				
Normal	31 (29.5)	7 (22.6)	24 (32.4)	0.28
Underweight	61 (58.1)	22 (71.0)	39 (52.7)	
Overweight	10 (9.5)	1 (3.2)	9 (12.2)	
Obese	3 (2.9)	1 (3.2)	2 (2.7)	
National Capital Region	36 (34.3)	10 (32.3)	26 (35.1)	0.78
Baseline CXR				
Cavitary	24 (22.9)	4 (12.9)	20 (27.0)	0.15
Non-cavitary	47 (44.8)	18 (58.1)	29 (39.2)	
Unknown	34 (32.4)	9 (29.0)	25 (33.8)	
Smear grade <sup>^</sup>				
Negative or scanty	24 (22.9)	4 (12.9)	20 (27.0)	0.07
(1+)	23 (21.9)	6 (19.4)	17 (23.0)	
(2+)	19 (18.1)	10 (32.3)	9 (12.2)	
(3+)	39 (37.1)	11 (35.5)	28 (37.8)	
Previous TB treatment	102 (97.1)	30 (96.8)	72 (97.3)	0.88
<b>Heteroresistance</b>				
Macro (>10%)	21 (20.0)	21 (67.7)	-	
Micro (≤10%)	7 (6.7)	7 (22.6)	-	
Rare	3 (2.9)	3 (9.7)	-	
Heteroresistance to:				
Fluoroquinolones	26 (24.8)	26 (83.9)	-	
Second-line injectables	2 (1.9)	2 (6.5)	-	
Isoniazid	2 (1.9)	2 (6.5)	-	
Rifampicin	1 (1.0)	1 (3.2)	-	
Streptomycin	4 (3.8)	4 (12.9)	-	
>1 resistant population	5 (4.8)	3 (9.7)	2 (2.7)	0.13

XDR: Extensively drug-resistant, TB: Tuberculosis, CXR: Chest x-ray, IQR: interquartile range

\*p-value for the association of clinical factor with heteroresistance, using chi-squared test for categorical variables and the Wilcoxon rank-sum to compare median age

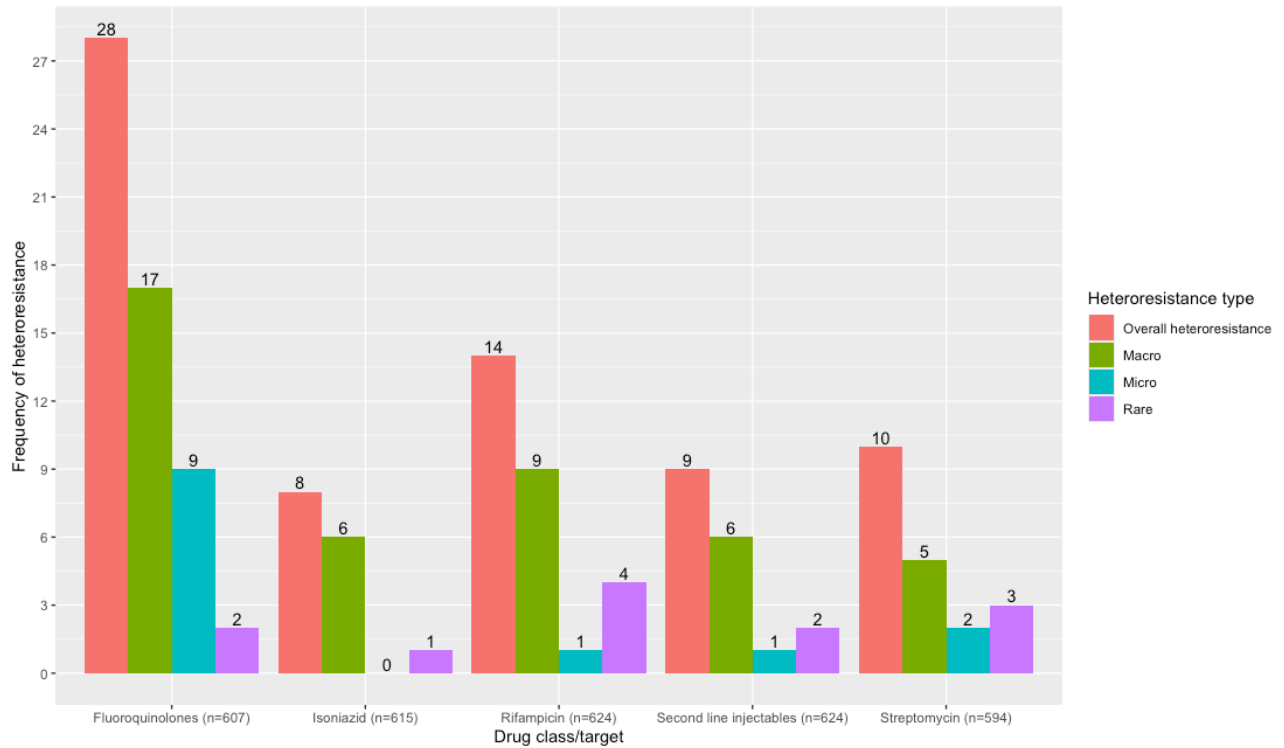
<sup>^</sup>Highest pre-treatment smear grade



**Table S6. Distribution of degree of heteroresistance based on the phenotypic susceptibility test**

	Total of each degree of heteroresistance	Phenotypic resistance n (%)	Phenotypic susceptibility n (%)
<b>Macro</b>	43	39 (65.0)	4 (44.4)
<b>Micro</b>	13	11 (18.3)	2 (22.2)
<b>Rare</b>	13	10 (16.7)	3 (33.3)
<b>Total</b>	69	60	9

**Figure S1. Frequency of heteroresistance by drug class/target**



**Table S7. Hazard of unfavorable outcome associated with heteroresistance in multiple imputation analysis**

	N	Unadjusted Hazard Ratio (95% CI)	Adjusted Hazard Ratio* (95% CI)	P-value*
<b>Heteroresistance</b>				
<b>MDR TB</b>	379	1.26 (0.31, 5.23)	1.56 (.35, 6.88)	0.59
<b>MDR plus SLIR</b>	140	0.83 (0.11, 6.24)	1.14 (0.13, 9.87)	0.90
<b>Pre-XDR</b>	105	0.74 (0.29, 1.87)	0.64 (0.24, 1.74)	0.39

\* Adjusted for known demographic and clinical risk factors with significance p=0.2 (age, region of the Philippines, cavitory chest X-ray, treatment history, body mass index).

MDR: multi-drug resistant; SLIR: second-line injectable resistant; XDR: extensively drug resistant.