

pncA mutations in MDR-TB strains from Panama

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MDR-TB in Panama

- Culture-based methods used for DST



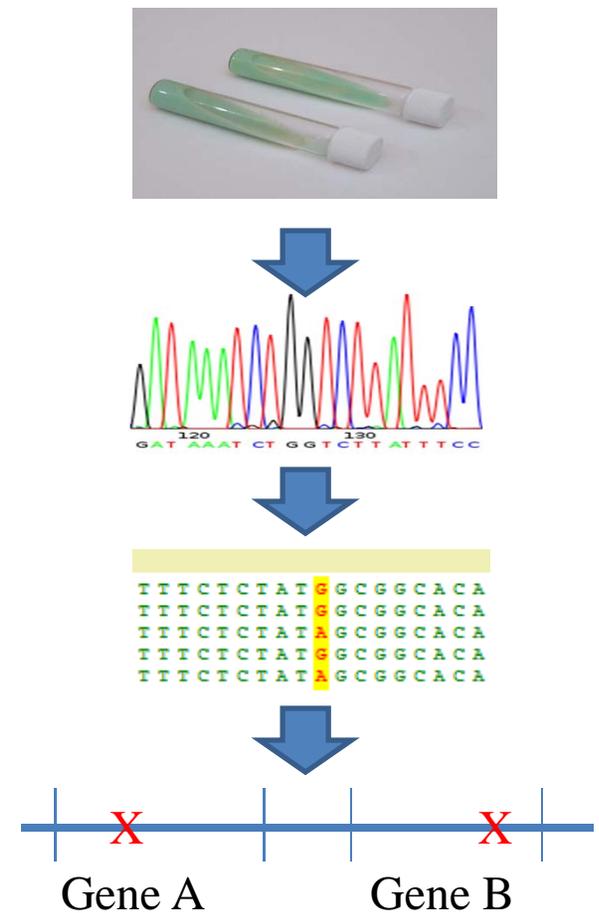
- MDR-TB in Panama
 - 2.0% (0-6.0%) of new cases
 - 12.1% (0–28.3%) of previously treated cases

(WHO, M/XDR TB 2010 report on surveillance and response)

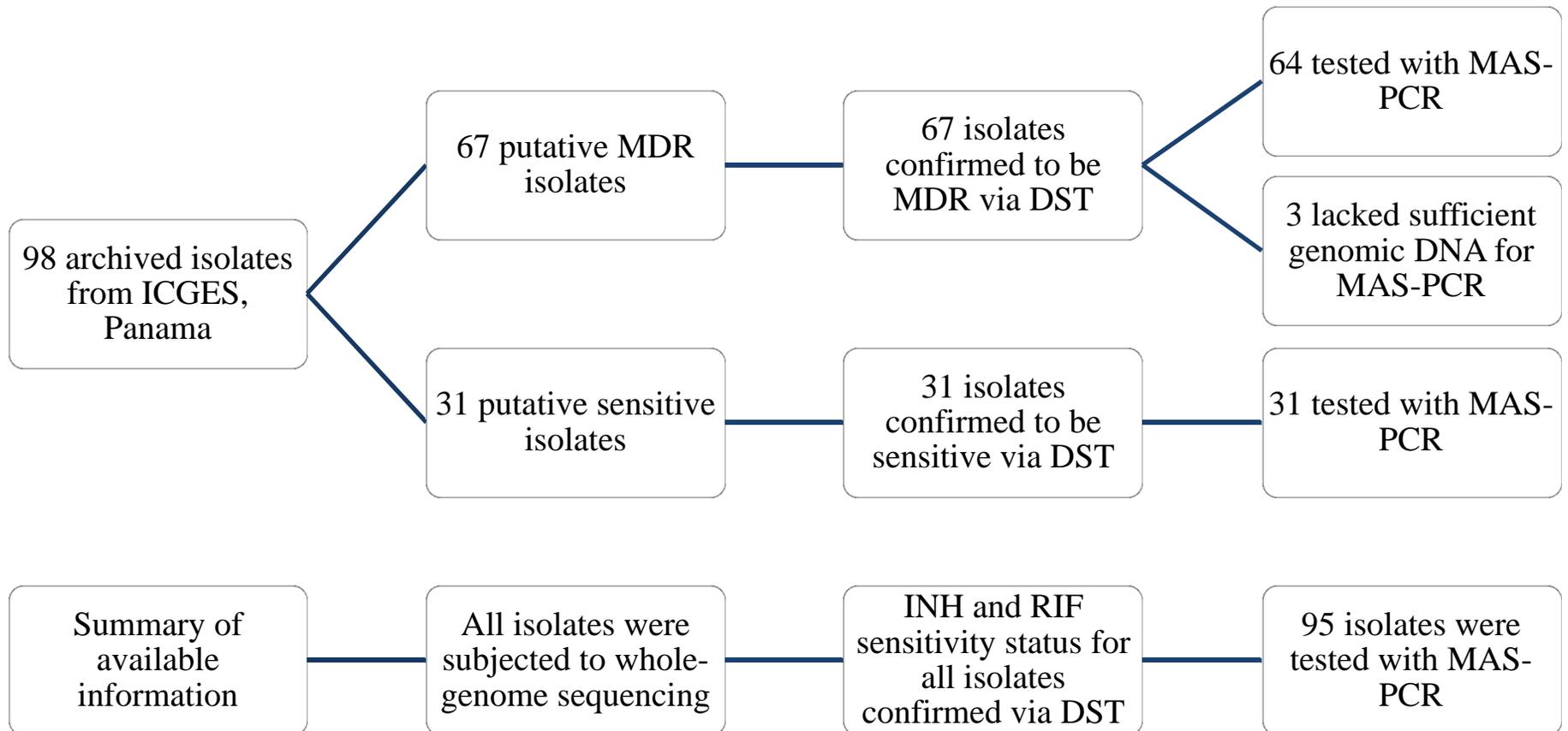
- The prevalence of individual genetic mutations associated with drug resistance among MDR-TB clinical isolates from Panama is unknown.

Characterization of *Mtb* drug-resistance mutations in Panama

- Archived DST-confirmed MDR-TB clinical isolates from Panama (ICGES)
- Whole genome sequencing performed at Texas A&M University (TAMU)
- Multiple Sequence Alignment
- Identification of mutations associated with drug resistance and calculation of mutation frequencies



Study Design



Geographic distribution of drug-sensitive strains



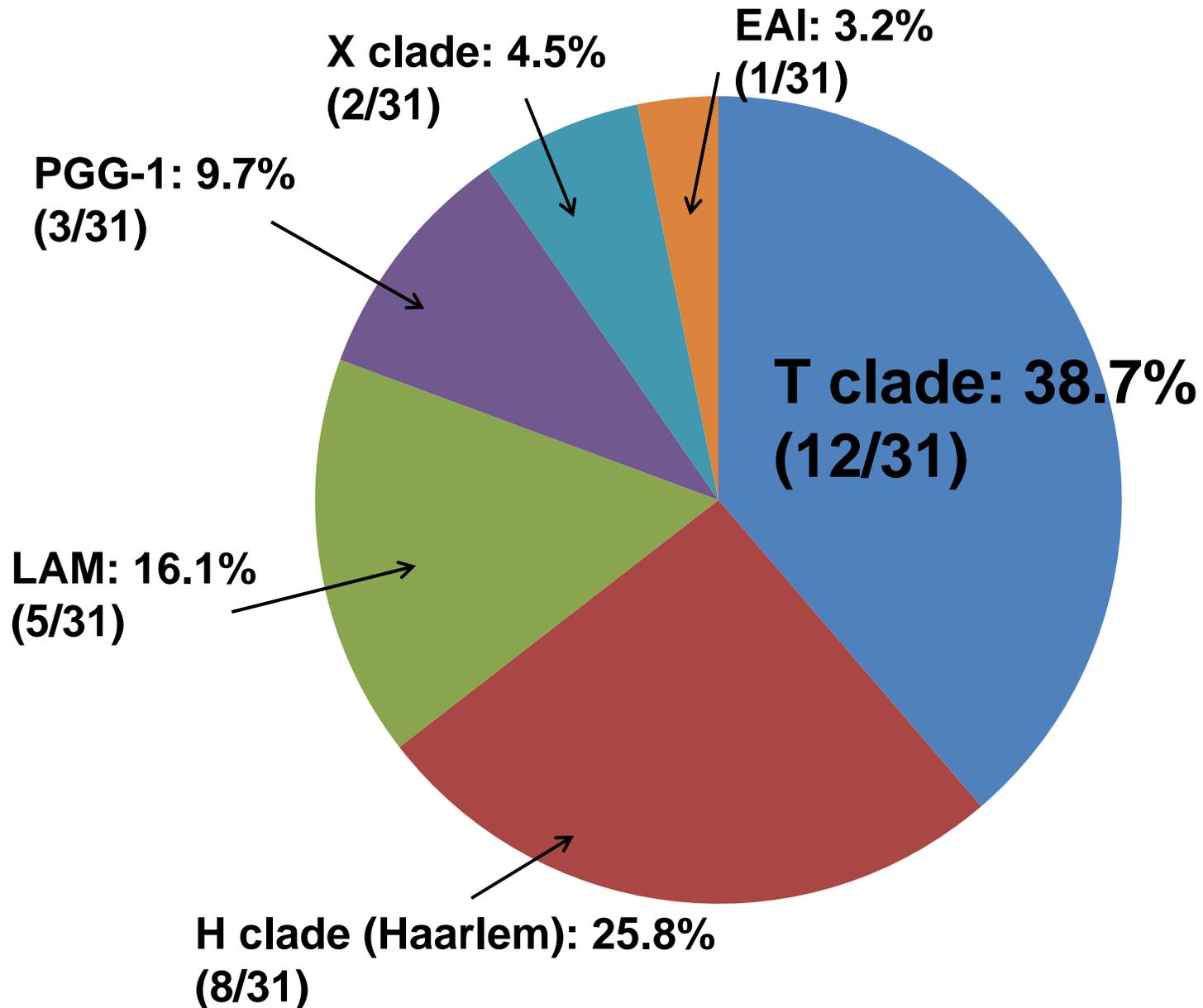
- Panamá Metro area: 84% (26/31)
- Colón: 13% (4/31)

Geographic distribution of MDR-TB strains

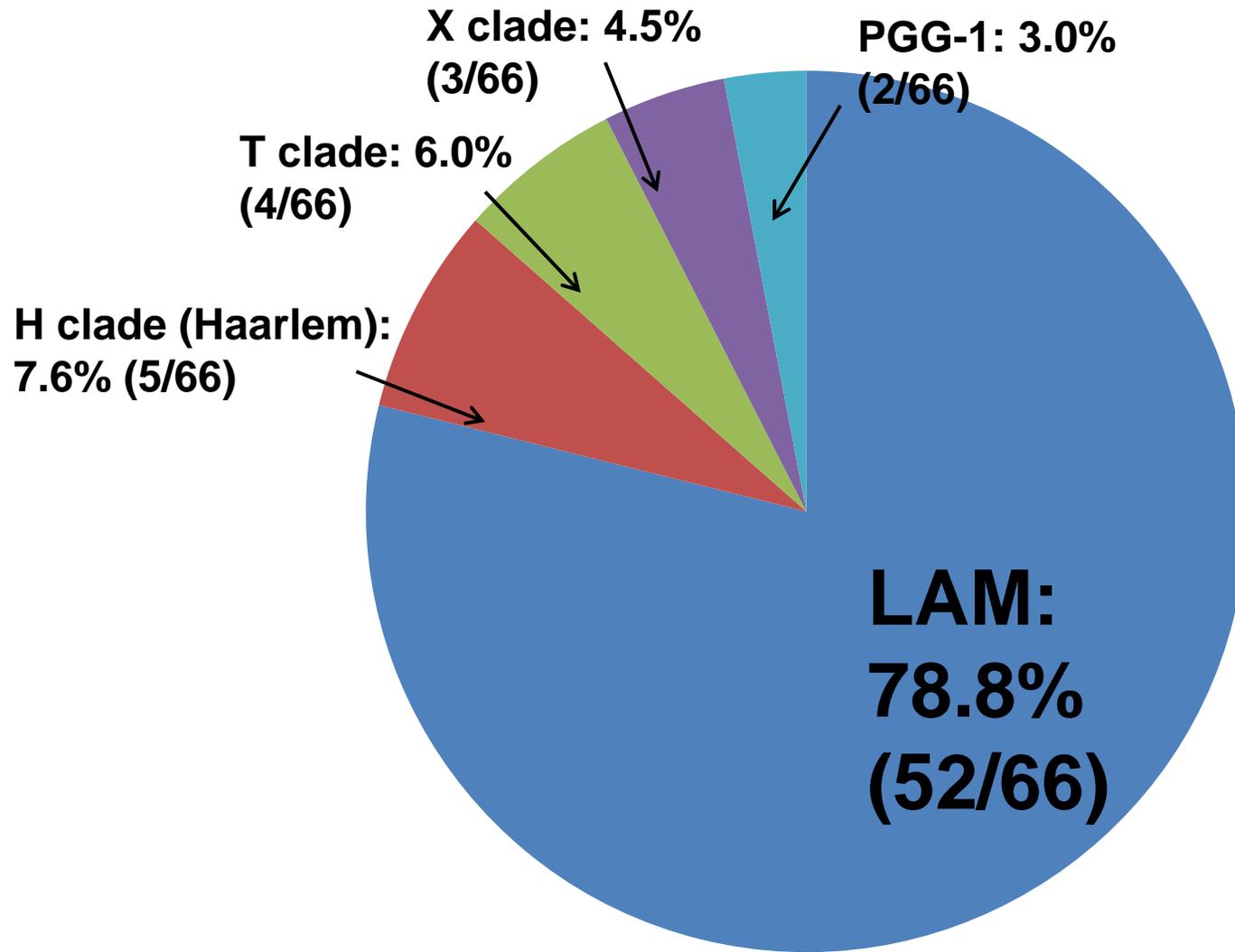


- Panamá Metro area: 34% (23/67)
- Colón: 33% (22/67)
- Chiriquí: 15% (10/67)

Genealogy: drug-sensitive strains

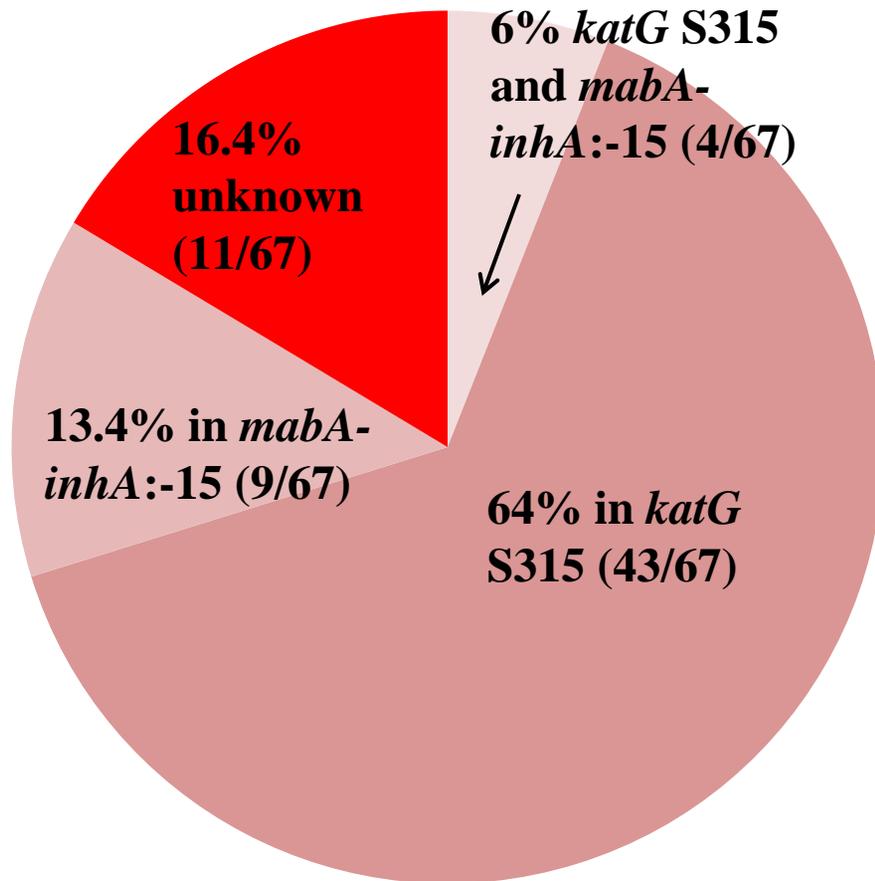


Genealogy: MDR-TB strains

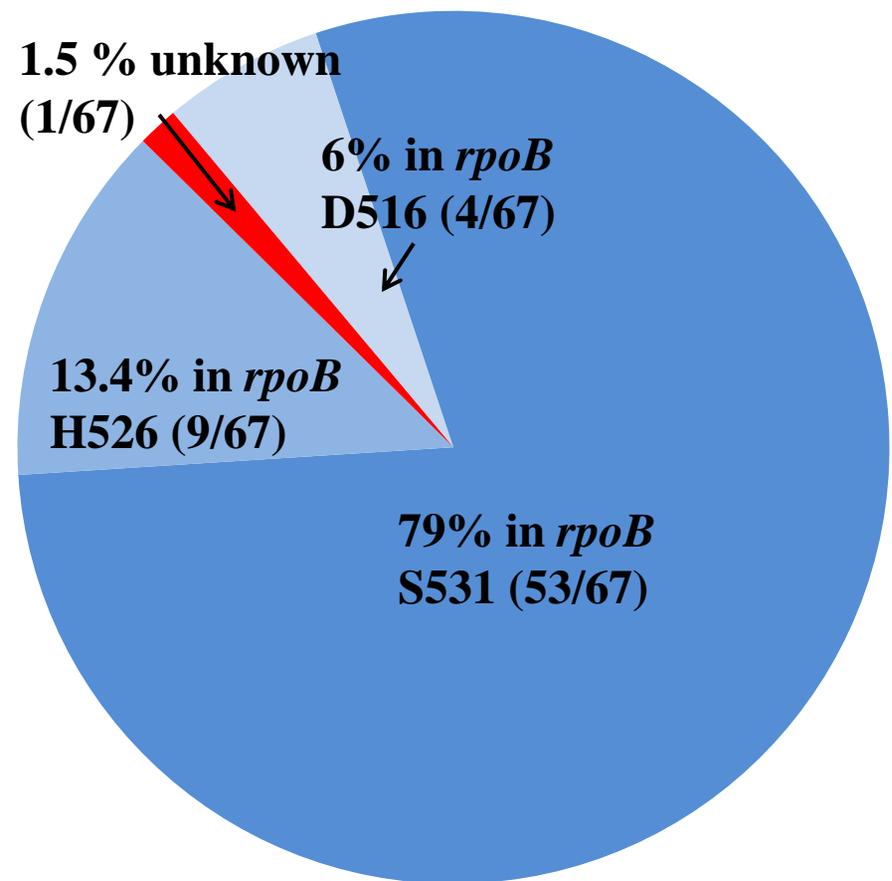


Mutations associated with INH and RIF resistance

INH Resistance



RIF Resistance



Frequency of mutations associated with INH and RIF resistance

Drug Resistance	Genes	Mutations	Number of Resistant Strains (Frequency out of 67 Strains)	Reported Mutation Frequency
INH	<i>katG</i>	S315T/G	47 (70.1%)	50-95%
	<i>inhA</i> promoter	c-15t	13 (19.4%)	8-43%
RIF	<i>rpoB</i>	D516F/V	4 (6.0%)	95%
		H526D/Y	9 (13.4%)	
		S531L	53 (79.1%)	

Novel mutations potentially accounting for INH resistance

Candidate Mutations (No. of strains in which mutation is found)	Predicted Outcome
3' 320 bp of <i>katG</i> deleted (1)	Truncated KatG
<i>katG</i> W198* (1)	Truncated KatG
<i>katG</i> Q722* (1)	Truncated KatG
<i>katG</i> D94G and <i>ahpC</i> P44R (2)	Inactivated KatG
500kb duplication spanning <i>Rv3177-Rv3573c</i> (1)	Duplicated Nat, increased INH inactivation

MDR-TB and PZA resistance: Global prevalence

Country	Percent PZA resistance among MDR-TB isolates
Japan	53%¹
South Africa	54%²
Peru	59%³
Cambodia	47%⁴

¹Ando H et al. *Clin Microbiol Infect* 2010; 16: 1164–1168.

²Louw GE et al. *Int J Tuberc Lung Dis* 2006; 10: 802–807.

³Saravia JC et al. *Int J Tuberc Lung Dis* 2005; 9: 421–429.

⁴Pierre-Audigier C et al. *Int J Tuberc Lung Dis* 2012; 16: 221–223.

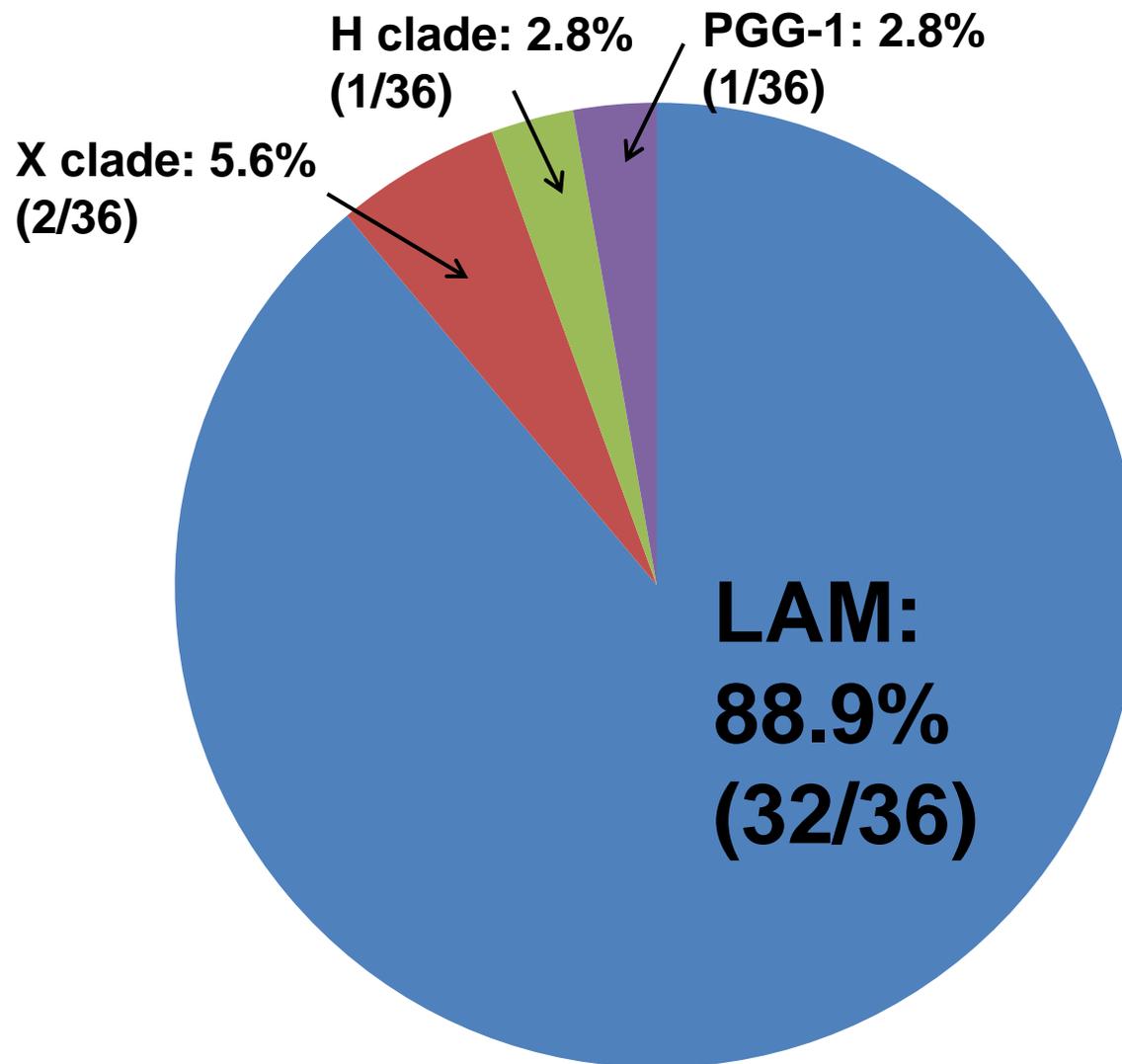
Association of MDR with *pncA* mutations

Sensitivity to INH/RIF	# strains with mutations in <i>pncA</i> (%)
Yes	0/31 (0%)
No (MDR-TB)	33/67 (49.3%)

pncA mutations in 33 MDR-TB strains

Mutations in <i>pncA</i> gene	Number of strains (%)	Reported in association with PZA resistance (# of strains)
V9A (GTG→GCG)	1 (3%)	Yes (1)
F13V (TTC→GTC)	1 (3%)	No – F13S (1)
T47A (ACC→GCC)	1 (3%)	Yes (7)
K48T (AAG→ACG)	3 (9%)	No – K48E (1)
H57Y (CAC→TAC)	3 (9%)	Yes (1)– H57D (16)
H71T (CAT→TAT)	1 (3%)	No– H71 (14)
T76P (ACT→CCT)	1 (3%)	Yes (9)
A79E (GCG→GAG)	4 (12%)	No– A79V (1)
F94 Insertion (+A)	1 (3%)	No
G108E (GGA→GAA)	1 (3%)	No– G108R (2)
W119R (TGG→CGG)	1 (3%)	Yes (3)
W119STOP (TGG→TGA)	5 (15%)	Yes (1)
Q122STOP(CAA→TAA)	1 (3%)	No
G132 Insertion (+A)	8 (24%)	No
A134V (GCC→GTC)	1 (3%)	Yes (6)
A146V(GCG→GTG)	1 (3%)	Yes (1)– A146 (3)
L159P (CTG→CCG)	1 (3%)	Yes (4)
A165I (GCC→ATC)	1 (3%)	No

Familial distribution of strains with *pncA* mutations



pncA mutations in FQ-resistant strains

PZA resistance has been reported in > 90% of FQ-resistant MDR strains[†].

Non-synonymous SNPs or indels were found in 4/6 MDR strains with significant *gyrA* mutations (A90V, D94G).

Conclusions

- Half of all MDR-TB, but none of the INH/RIF-sensitive, strains from Panama were found to have *pncA* mutations.
- The LAM family was highly represented among strains with *pncA* mutations.
- *pncA* mutations were distributed throughout the gene, with 15/36 mutations previously reported to be associated with PZA resistance.
- The most common mutation (G132 insertion) was found in 8 clustered LAM strains, accounting for a quarter of cases.

Acknowledgments

Johns Hopkins Center for
TB Research

Bing Shao Chia

Edith Torres-Chavolla, PhD

Dalin Rifat, PhD

Lee Klinkenberg, PhD

Texas A&M University

Thomas Ioerger, PhD

James Sacchettini, PhD

ICGES, Panama

Fedora Lanzas

Juan M. Pascale, PhD