Antibiotic Resistance and Single-Nucleotide Polymorphism Cluster Grouping Type in a Multinational Sample of Resistant *Mycobacterium tuberculosis* Isolates

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A single-nucleotide polymorphism-based cluster grouping (SCG) classification system for *Mycobacterium tuberculosis* was used to examine antibiotic resistance type and resistance mutations in relationship to specific evolutionary lineages. Drug resistance and resistance mutations were seen across all SCGs. SCG-2 had higher proportions of *katG* codon 315 mutations and resistance to four drugs.

Isoniazid (INH) is an effective agent for treatment of infections with *Mycobacterium tuberculosis*. Increases in INH-resistant and multidrug-resistant tuberculosis jeopardize drug effectiveness (7, 24), and development of INH resistance is often a first step in multidrug resistance (2, 8). Mutations in specific genes have been linked to INH-related resistance (10, 11), including *katG* (26), *inhA* (14), codon 315 of *katG* (*katG*315) (13, 16, 19, 23), *ahpC* (20), the *inhA* open reading frame and promoter (14, 17, 25), and *ndh* (22).

Recent studies of drug-resistant *M. tuberculosis* have found associations among *M. tuberculosis* strains, drug resistance, and specific gene mutations. *M. tuberculosis* strains belonging to the Beijing family were associated with drug resistance in Iran, Afghanistan, and Russia (6, 12, 15, 18), although not in Venezuela (5). These associations have also been supported through genetic laboratory studies (1, 21). Thus, it is possible that specific types of drug resistance or drug resistance mutations might occur more commonly in certain evolutionary lineages of *M. tuberculosis*.

The single-nucleotide polymorphism (SNP) cluster grouping (SCG) classification system defined in reference 8 gives rise to seven phylogenetically distinct groups and three subgroups that can be used to infer an evolutionary pattern in *M. tuberculosis*. Here, we analyze 428 *M. tuberculosis* isolates resistant to at least INH collected across 10 countries and report the prevalence of various INH resistance-associated mutations and prevalences of resistance to two, three, and four drugs according to the major SCG-defined phylogenetic lineages of *M. tuberculosis*.

*M. tuberculosis* isolates resistant to at least INH were obtained from laboratories in major medical centers in Australia, Colombia, India, Mexico, New York City, Spain, and Texas (Table 1). The study population and sample selection have been described previously (10, 11), with each collection site

<table>
<thead>
<tr>
<th>Location</th>
<th>No. (%) of isolates</th>
<th>M. bovis</th>
<th>M. tuberculosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SCG-1   SCG-2   SCG-3a SCG-3b SCG-3c SCG-4 SCG-5 SCG-6a SCG-6b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Australia</td>
<td>0       9 (30.0) 11 (36.7) 3 (10.0) 3 (10.0) 0 0 3 (10.0) 0 1 (3.3)</td>
<td>30 (100)</td>
<td></td>
</tr>
<tr>
<td>Colombia</td>
<td>0       0 0 1 (0.7) 33 (23.1) 1 (0.7) 6 (4.2) 84 (58.7) 17 (11.9) 1 (0.7)</td>
<td>143 (100)</td>
<td></td>
</tr>
<tr>
<td>India</td>
<td>0       2 (6.7) 1 (3.3) 23 (76.7) 1 (3.3) 0 0 2 (6.7) 1 (3.3) 0</td>
<td>30 (100)</td>
<td></td>
</tr>
<tr>
<td>Mexico</td>
<td>2 (1.6) 0 1 (0.8) 1 (0.8) 44 (35.8) 11 (8.9) 14 (11.4) 31 (25.2) 15 (12.2) 4 (3.3)</td>
<td>123 (100)</td>
<td></td>
</tr>
<tr>
<td>New York City</td>
<td>0       1 (3.9) 17 (65.3) 0 2 (7.7) 1 (3.9) 0 2 (7.7) 3 (11.5) 0</td>
<td>26 (100)</td>
<td></td>
</tr>
<tr>
<td>Spain</td>
<td>0       0 0 0 1 (0.1) 0 0 8 (72.7) 2 (18.2) 0</td>
<td>11 (100)</td>
<td></td>
</tr>
<tr>
<td>Texas</td>
<td>0       5 (7.7) 8 (12.3) 38 (8.9) 28 (6.5) 92 (21.5) 18 (4.2) 26 (6.1) 146 (34.1) 53 (12.4) 428 (100)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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isolates were tested for virtually all SNP mutations in the *M. tuberculosis* katG, *kasA*, *mabA*, *inhA*, *oxyR*, *ahpC*, and *ndh* genes found to be associated with INH resistance in published studies (11). A total of 204 INH resistance-associated alleles were detected (9), with confirmatory testing carried out on alleles, identified mutations, and drug resistance.

Strain types are reported in terms of SCG grouping, based on observed genomic level clustering among identified SNPs (8). SCG assignment was performed by testing each isolate for nine SNPs previously determined to replicate larger SNP-based phylogeny, as described in references 3 and 4.

The genetic, resistance, and SCG data from each set of country-specific isolates were entered into a common database. Prevalence was reported by SCG type, country, selected genes, and resistance type. Chi-square tests of differences in proportions were employed as appropriate. The STATATA statistical package was used for all calculations.

The complete sample was tested for 240 alleles previously reported to be associated with INH resistance. Country-specific breakdowns by various types of resistance can be found in references 10 and 11. The overall prevalence of SCG groups is given in Table 1. The Beijing family (strongly associated with *M. bovis*) had all mutations of interest. SCG-1 and SCG-2 had the rarest (excluding SCG-5) mutations. SCG-1 and SCG-3b had the highest proportion of mutations in *katG*. Mutations in *inhA* and *ahpC* promoters were found in all SCGs with *n* > 17.

To examine antibiotic resistance on a cumulative scale, we examined resistance within each SCG classification type in relation to resistance to one, two, or more antibiotics. All SCG types (excepting *M. bovis*) had at least 46% of isolates with resistance to one or two of these antibiotics. The range across all SCG types was 46% to 82%. SCG-2 had the highest prevalence (29%) of resistance to all four antibiotics.

This study provides insights into the associations among drug resistance in *M. tuberculosis*, gene mutation, and genomic SCG-based phylogenetic lineages, utilizing a large number of resistant isolates and examining patterns of relationships across SCG classification type, resistance, gene mutation, and country.

The sample contained 8.9% SCG-2 (Beijing) isolates, similar to Asian samples (18) and comparable to other SCG types, except SCG-3b, SCG-5, and SCG-6a. SCG-2 isolates accounted for more than 10% of the KatG and KatG315 mutations in this study. Most mutations were prevalent in all SCGs.

The prevalence of resistance was high across all SCG types. Table 3 shows that SCG-2 had the highest prevalence of resistance to all antibiotics, but all SCG types display high levels of resistance to one and two antibiotics. SCG-6a displayed a high prevalence of isolates resistant to all four antibiotics. As the SCG classification reflects regions, the presence of antibiotic resistance in so many SCG classifications may reflect several ongoing evolutionary processes and implies a need to maintain a broad perspective on *M. tuberculosis* antibiotic resistance.


<table>
<thead>
<tr>
<th>SCG-1</th>
<th>SCG-2</th>
<th>SCG-3a</th>
<th>SCG-3b</th>
<th>SCG-3c</th>
<th>SCG-4</th>
<th>SCG-5</th>
<th>SCG-6a</th>
<th>SCG-6b</th>
<th>Total</th>
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<tr>
<td>1</td>
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<td>12 (70.59)</td>
<td>12 (31.58)</td>
<td>12 (42.86)</td>
<td>26 (28.26)</td>
<td>8 (44.44)</td>
<td>8 (30.77)</td>
<td>28 (19.18)</td>
<td>14 (26.42)</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>2 (11.76)</td>
<td>10 (26.32)</td>
<td>9 (32.14)</td>
<td>30 (32.61)</td>
<td>4 (22.22)</td>
<td>12 (46.15)</td>
<td>39 (26.71)</td>
<td>16 (30.19)</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>2 (11.76)</td>
<td>5 (13.16)</td>
<td>6 (21.43)</td>
<td>21 (22.83)</td>
<td>5 (27.78)</td>
<td>4 (15.38)</td>
<td>53 (36.30)</td>
<td>10 (18.87)</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>1 (5.88)</td>
<td>11 (28.95)</td>
<td>1 (3.57)</td>
<td>15 (16.3)</td>
<td>1 (5.56)</td>
<td>2 (7.69)</td>
<td>26 (17.81)</td>
<td>13 (24.53)</td>
</tr>
</tbody>
</table>

Total: 2 (17) | 10 (38) | 38 (100) | 28 (100) | 92 (100) | 18 (100) | 26 (100) | 146 (100) | 53 (100) | 8 (100) | 428 (100)

**Limitations.** This study analyzed *M. tuberculosis* isolates for mutations associated with INH resistance in previous studies. The results therefore may not be completely representative of mutations relevant to resistance. The overall patterns observed in the SCG classification may have been restricted by the method of sample selection, but the size and diversity of the sample make this unlikely.

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**REFERENCES**


