Infecting Drug Resistance of *Mycobacterium tuberculosis* Isolates in a Medical Center in Northern Taiwan

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**Background/Purpose:** This retrospective study was conducted to evaluate the drug resistance patterns of *Mycobacterium tuberculosis* in a medical center in northern Taiwan between 2003 and 2004 in comparison to those reported in 1990–1992.

**Methods:** A total of 611 non-duplicate *M. tuberculosis* isolates from culture-proven tuberculosis (TB) cases were tested for drug susceptibility against five first-line anti-TB drugs in a clinical mycobacterial laboratory using the agar proportional method for isoniazid (INH), rifampicin (RIF), ethambutol (EMB), and streptomycin (SM). The Wayne assay, which measures the activity of pyrazinamide (PZA), was used for PZA susceptibility testing.

**Results:** Of 611 patients, including 510 males and 101 females, 70.2% of patients were older than 65 years. A total of 339 isolates (55.5%) were resistant to one or more drugs. Isolates from patients aged <25 years showed a significantly higher drug resistance rate (79.2%) compared with other age groups (*p* = 0.0312). Single-drug resistance was observed in 97 (15.9%) of all isolates. Monoresistance to PZA (8.0%) was most frequent, followed by INH (5.1%), RIF (0.5%), EMB (1.6%), and SM (0.7%). Among the polydrug resistant isolates (PDR-TB), resistance rates were 35.5% for INH and 27.0% for RIF. One hundred and fifty-nine isolates (26.0%) were resistant to both INH and RIF (multidrug-resistant [MDR] TB); 94.6% of RIF-resistant isolates were also resistant to INH. The overall drug resistance rates and percentages of PDR-TB and MDR-TB increased over the 12-year study period (*p* < 0.001). Based on medical records, primary cases were identified in 486 (84.7%) out of 574 patients, and resistance to any drug was identified in 268 (55.1%) patients, of which 130 (26.7%) were MDR-TB. Among the 88 with recurrent TB, 54 (61.4%) were resistant to at least one drug, and MDR-TB was identified in 29 (33.0%) patients. A history of previous anti-TB therapy was a significant factor for overall drug resistance, PZA monoresistance, PDR-TB, and MDR-TB (*p* < 0.001).

**Conclusion:** The emergence of *M. tuberculosis* isolates resistant to anti-TB agents in this hospital, and in particular among young patients, is alarming. Strict measures to control and prevent drug-resistant TB are urgently needed. [J Formos Med Assoc 2008;107(3):259–264]

**Key Words:** drug resistance, *Mycobacterium tuberculosis*, Taiwan

Tuberculosis (TB) remains a common infection and an important cause of death throughout the world. In 1993, the World Health Organization (WHO) declared TB a global emergency. TB is the leading cause of death attributable to a single infectious pathogen. One-third of the world’s populations are at risk of developing the disease. In countries confronted with the human immunodeficiency virus (HIV) epidemic, the overlap of these two populations leads to a rapid acceleration...
of active TB and of the emergence of multidrug-resistant (MDR) TB. In Taiwan, the annual incidence of TB fell from 64.9/100,000 in 1998 to 62.7/100,000 in 2000, but increased to 64.8/100,000 in 2001. High drug resistance rates have been continually reported in Taiwan. These figures raised concerns that TB remains a potential public health problem in Taiwan.

An effective national program for the control of TB relies upon case-finding and treatment to eliminate sources of infection. Unfortunately, resistance of Mycobacterium tuberculosis has been encountered against all anti-TB drugs. The major risk factors for the emergence of drug resistant TB are patient noncompliance and inappropriate administration of drugs by clinicians. Therefore, the pattern of drug resistance in the health institution is recognized as a good indicator of the efficiency of the treatment service.

The objectives of the present study were: (1) to determine the susceptibility of M. tuberculosis isolates to first-line anti-TB drugs during the period 2003–2004; and (2) to compare the drug susceptibility patterns of isolates with the results of earlier reports from this institution in 1990–1992.

Method

The present analysis is based on the results of susceptibility testing of M. tuberculosis strains isolated at Taipei Veterans General Hospital from January 2003 to December 2004. Taipei Veterans General Hospital is a 2901-bed teaching hospital in northern Taiwan responsible for the diagnosis and treatment of patients with acute and chronic diseases, including TB.

Sputum samples were cultured on Löwenstein-Jensen media. Non-tuberculous mycobacteria were excluded based on results of biochemical tests of the niacin test and nitrate test, colony morphology and pigmentation, and growth rate on solid media. All enrolled cases were of culture-proven M. tuberculosis disease. Only the initial isolate of a patient from whom multiple specimens were received was tested for drug susceptibility by the conventional proportion method with Middlebrook 7H11 agar plate media. As the low pH is inhibitory to the growth of M. tuberculosis, which may not precisely detect pyrazinamide (PZA) resistant isolates, the Wayne assay, which measures the activity of PZA, was used for PZA susceptibility testing. Susceptibility was tested in vitro for the following drugs and concentration (µg/mL): isoniazid (INH), 0.2, 1.0; ethambutol (EMB), 5.0, 10.0; rifampicin (RIF), 1.0, 5.0; streptomycin (SM), 2.0, 10.0; PZA, 25, 50; and with appropriate controls.

Organisms were considered resistant to a given drug if any growth of 1% or more above the drug-free control was observed in the presence of the critical concentration and/or higher concentration of the drug tested. MDR-TB refers to organisms that are resistant to two or more anti-TB drugs, including RIF and INH. Polydrug resistant (PDR) TB refers to the pattern of drug resistance to several agents except MDR-TB. The species identification of the isolates was based on standard microbiological tests: colony morphology, acid-fast staining and biochemical tests. Overall resistance rate was defined as the prevalence of drug resistance among all cases of TB, regardless of prior drug treatment. Based on clinical history, resistance among new cases was defined as primary resistance, while resistance among previously treated cases was defined as acquired resistance.

Statistical analysis of categorical variables was performed by χ² test or Fisher’s exact test. A p value of less than 0.05 was considered statistically significant.

Results

During the 2-year study period, based on one isolate per patient, M. tuberculosis isolates from 611 patients in Taipei Veterans General Hospital were available for analysis. Patients included 510 males and 101 females, 429 (70.2%) of whom were older than 65 years. The proportion of overall drug resistance was higher in females (63/101, 62.4%) than in males (276/510, 54.1%), although the difference was not significant.
The overall drug resistant rate was significantly higher in those aged <25 years (79.2%; \( p = 0.0312 \)) compared to those aged 25–45 years (61.4%) and >65 years (55.2%) (Table 1). Single-drug resistance was present in 97 (15.9%) of all isolates, with a higher rate for PZA (8.0%), followed by INH (5.1%). Resistance to RIF, EMB and SM alone was 0.5%, 1.6% and 0.7%, respectively (Table 2).

Based on the medical records, primary cases were identified in 486 (84.7%) out of 574 patients, while those in the years 1990–1992 were 53.9%. Among the 486 primary cases, resistance to any drug was identified in 268 (55.1%) patients, in whom 130 (26.7%) were MDR-TB. Among the 88 with recurrent TB, 54 (61.4%) were resistant to at least one drug, and MDR-TB was identified in 29 (33.0%) patients. A history of previous anti-TB therapy was significantly associated with overall drug resistance, PZA monoresistance, and rates for both PDR-TB and MDR-TB (\( p < 0.001 \)) (Table 2).

During the 12-year study period, a significant increase in drug resistance was observed, comparing the years 1990–1992 (22.6%) to 2003–2004 (55.5%) (\( p < 0.001 \)) (Figure).

### Table 1. Drug resistance rate by age and prior treatment history, 2003–2004

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Overall resistance (( n = 574 ))</th>
<th>Drug resistance based on treatment history (( n = 574 ))</th>
<th>Primary</th>
<th>Acquired</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;25</td>
<td>19/24 (79.2%)*</td>
<td>5/7 (71.4%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>25–45</td>
<td>35/57 (61.4%)</td>
<td>26/41 (63.4%)</td>
<td>6/9 (66.7%)</td>
<td></td>
</tr>
<tr>
<td>46–65</td>
<td>48/101 (47.5%)</td>
<td>40/88 (45.5%)</td>
<td>8/16 (50%)</td>
<td></td>
</tr>
<tr>
<td>≥65</td>
<td>237/429 (55.2%)</td>
<td>197/350 (56.3%)</td>
<td>40/63 (63.5%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>339/611 (55.5%)</td>
<td>268/486 (55.1%)</td>
<td>54/88 (61.4%)</td>
<td></td>
</tr>
</tbody>
</table>

*\( p = 0.0312 \) (Fisher’s exact test), compared with rates of other age groups.

### Table 2. Comparison of drug resistant patterns of *Mycobacterium tuberculosis* isolates in two time periods

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Single drug resistance</td>
<td></td>
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</tr>
<tr>
<td>INH</td>
<td>22 (3.4%)</td>
<td>31 (5.1%)</td>
<td>0.134</td>
<td>26 (5.4%)</td>
<td>1 (1.1%)</td>
<td></td>
</tr>
<tr>
<td>RIF</td>
<td>8 (1.2%)</td>
<td>3 (0.5%)</td>
<td>0.227†</td>
<td>2 (0.4%)</td>
<td>0</td>
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</tr>
<tr>
<td>EMB</td>
<td>16 (2.5%)</td>
<td>10 (1.6%)</td>
<td>0.305</td>
<td>2 (0.4%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>SM</td>
<td>18 (2.8%)</td>
<td>4 (0.7%)</td>
<td>0.004†</td>
<td>4 (0.8%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>PZA</td>
<td>–</td>
<td>49 (8.0%)</td>
<td>–</td>
<td>39 (8.0%)</td>
<td>10 (11.4%)†</td>
<td></td>
</tr>
<tr>
<td>Polydrug resistance</td>
<td></td>
<td></td>
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<tr>
<td>INH at least</td>
<td>74 (11.4%)</td>
<td>217 (35.5%)</td>
<td>&lt;0.001</td>
<td>202 (41.6%)</td>
<td>42 (47.7%)†</td>
<td></td>
</tr>
<tr>
<td>RIF at least</td>
<td>61 (9.4%)</td>
<td>165 (27.0%)</td>
<td>&lt;0.001</td>
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<tr>
<td>Multidrug resistance</td>
<td></td>
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<tr>
<td>INH-RIF at least</td>
<td>54 (8.3%)</td>
<td>159 (26.0%)</td>
<td>&lt;0.001</td>
<td>130 (26.7%)</td>
<td>29 (33.0%)†</td>
<td></td>
</tr>
<tr>
<td>INH-RIF-EMB-SM at least</td>
<td>24 (3.7%)</td>
<td>112 (18.3%)</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>INH-RIF-EMB-SM-PZA</td>
<td>–</td>
<td>30 (4.9%)</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any drug</td>
<td>147 (22.6%)</td>
<td>339 (55.5%)</td>
<td>&lt;0.001</td>
<td>268 (55.1%)</td>
<td>54 (61.4%)†</td>
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</tbody>
</table>

*Patients in whom previous anti-tuberculosis therapy was identified; †Fisher’s exact test; ‡\( p < 0.001 \) (Fisher’s exact test), compared with patients without previous treatment.
to 40.6% \((p < 0.001)\), from 10.6% to 27.5% \((p < 0.001)\), from 10.3% to 31.3% \((p < 0.001)\), from 9.7% to 29.0% \((p < 0.001)\) and from 8.3% to 26.0% \((p < 0.001)\), respectively.

The majority (94.6%) of RIF-resistant isolates were also resistant to INH, while 64.1% of INH-resistant isolates were also RIF-resistant. Thirty isolates (4.9%) were resistant to all five first-line agents. A comparison of overall drug resistance rates in different institutes in Taiwan is summarized in Table 3.

### Discussion

While the incidence of TB may be declining in the general community, the bacteriologic confirmation of the disease is increasingly important due to the changing and varied presentation of the disease and also due to the higher rates of drug resistance that are currently being detected. The use of combinations of anti-TB agents has not prevented drug resistance from occurring in Taiwan.\(^{17}\) The increasing prevalence of anti-TB drug resistance is a consequence of the unsatisfactory quality of case management of TB, including inadequately administered therapy based on outdated drug-susceptibility data. Therefore, it is very important to find out new TB cases as early as possible, treat them with a proper regimen and prevent dropout by directly observed therapy, thus preventing the emergence of MDR-TB. In addition, clinicians must have current knowledge of the local epidemiology of TB, and laboratories should maintain up-to-date drug susceptibility data on the local isolates of *M. tuberculosis*.

The incidence of TB in Taiwan has increased in recent years, and the prevalence of drug resistance in Taiwan parallels the worldwide situation for tuberculosis.\(^{3-8}\) The WHO’s global surveillance
for anti-TB drug resistance from 1994 to 1997 had reported high resistance to anti-TB agents in 35 countries, and there were repeated reports in the medical literature of MDR-TB in countries including Taiwan. In recent years, the infrastructures of TB control in Taiwan were changed to a centralized, government-budgeted and vertical TB control system. The clinician has responsibility for the treatment of TB in general health facilities. Therefore, routine surveys of drug resistance in referral hospitals are necessary to provide a guide to treatment recommendations, and to decrease the rate of anti-TB drug resistance.

In this study, we reported drug resistance rates to anti-TB drugs in 2003–2004 compared to those reported in 1990–1992 at Taipei Veterans General Hospital using a retrospective hospital-based surveillance. We found a marked increase in drug resistance rates of PDR-TB and MDR-TB in the past 12 years (p < 0.001) (Figure). Single resistance to specific drugs did not change significantly over time for all first-line anti-TB agents except the decreasing resistance rate to streptomycin, which may be due to streptomycin being no longer in use for primary treatment in Taiwan. Many factors contribute to the emergence of drug-resistant TB in this hospital, the main one being that this is a medical center. The increasing rate of primary cases in 2003–2004 compared to 1990–1992 (84.7% and 53.9%, respectively) indicated that the worst cases may be referred to this center, especially patients who had previously received anti-TB treatment. This suggests the possibility of changes in referral patterns to this hospital over the period of study. Secondly, there were 429 (70.2%) patients older than 65 years, which may result in a higher resistance rate due to the possibility of reactivation (Table 1). The overall drug resistance in this study differs significantly from those of recent reports in Taiwan (Table 3). However, drug susceptibility testing has its margins of limitations, varying from laboratory to laboratory and over time, while two concentrations are recommended for each antibiotic, so that results obtained by different laboratories or in different years will vary among these studies. In this study, the lower concentration was used. If anything, this may have caused a tendency to overestimate the prevalence of drug resistance in our study.

As with previous reports, patients younger than 65 years were found to have a higher rate of drug resistance. We also found that the number of isolates received from patients aged under 25 years was small (n = 24), but the drug resistance rate (79.2%) in this group was significantly higher than in the other age groups, which were 61.4% in those aged 25–45 years, and 55.2% in those aged > 65 years (Table 1). Such findings may suggest that certain groups of people are at a higher risk of acquiring drug resistance, such as younger patients. A high index of suspicion and prompt investigations in young patients may allow for earlier diagnosis and treatment of TB. More studies to delineate the effect of age on the presentation of patients with TB are needed.

MDR-TB is a major public health problem because treatment is complicated, cure rates are well below those for drug-susceptible TB, and patients may remain infectious for months or years, despite receiving the best available therapy. As shown in Table 2, a history of previous anti-TB therapy was significantly associated with overall drug resistance, PZA monoresistance, PDR-TB and MDR-TB. In this study, 94.6% of RIF-resistant isolates were also resistant to INH. Liaw et al also reported a MDR-TB rate of 83.7% among RIF-resistant isolates at a university hospital, so RIF resistance is predictive of MDR-TB. The use of rapid molecular methods to identify RIF resistance in cases with risk factors for MDR-TB is likely to prove highly effective in facilitating early detection of MDR cases. To reduce the problem of resistance for TB, we recommend the use of at least four drugs (INH, RIF, EMB, PZA) in the first 2 months. In a recent report from Taiwan, MDR-TB patients who received ofloxacin were more likely to be cured, and were less likely to die, fail or relapse. However, the role of new fluoroquinolones such as moxifloxacin in the treatment of MDR-TB needs to be evaluated.

In conclusion, although the results from this hospital laboratory are not representative of Taiwan
as a whole, the emergence of drug resistance over a 12-year period is a challenging issue for future TB management, and surveys of drug resistance should be repeated annually at Taipei Veterans General Hospital. Further studies are needed to confirm or refute the apparent high levels of resistance among patients with no previous treatment, as reported in the present study. It should be noted that 4.9% of isolates in this study were resistant to all five first-line drugs, which highlights the need for continuous monitoring of drug resistance to anti-TB drugs, and integrating patient treatment and follow-up with a more accessible primary health care system such as the Directly Observed Short Course program to improve poor compliance, which is an important factor in the development of drug resistance and treatment failure.22

References