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Drug resistance of *Mycobacterium tuberculosis* complex in central Taiwan

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The prevalence of drug resistance to *Mycobacterium tuberculosis* complex (MTBC) shows marked geographic difference and is the key to determining drugs of choice for the initial treatment of tuberculosis (TB). This retrospective study investigated the MTBC resistance rate and its contributing factors based on the review of medical records from a hospital in central Taiwan. TB culture and antimicrobial susceptibility test were performed using the BACTEC™ MGIT™ 960 System. Isoniazid, rifampin, ethambutol and streptomycin were tested. Molecular detection of MTBC using BDProbeTec™ ET kits was conducted in positive culture media containing acid-fast bacilli. Between July 2001 and June 2002, 974 (12.4%) strains of MTBC were isolated from 7892 clinical specimens from 513 patients. They included 348 males and 165 females with mean age of 66.1 ± 15.6 years and 63.4 ± 19.2 years, respectively. Sixty one percent of patients were older than 64 years of age. The overall resistance rate to 1 or more drugs was 22.4% (n = 115). The number of strains resistant to individual drugs was 86 (16.8%) to isoniazid, 25 (4.9%) to rifampin, 21 (4.1%) to ethambutol, and 63 (12.3%) to streptomycin. Twenty (3.9%) isolates were resistant to at least isoniazid and rifampin. A history of anti-TB treatment was associated with drug resistance (36.8% vs 20.6%, p=0.0056). Only 22 (4.3%) patients were tested for HIV antibodies and the results were all negative. The prevalence of resistance to anti-TB drugs remains high in Taiwan and is associated with a previous history of anti-TB treatment. Retreatment may contribute to an increased prevalence of multiple drug resistance.

Key words: Drug resistance, microbial sensitivity tests, multidrug-resistant tuberculosis, *Mycobacterium tuberculosis* complex

Tuberculosis (TB) remains the world's leading cause of death from a single infectious disease. The World Health Organization (WHO) report received notification of more than 3.67 million cases of TB (61 per 100,000 population of the whole world) in the year 2000. The WHO estimated that among approximately 8.74 million new cases in 2000, notification of only 42% was received. Of the reported cases, 1.02 million (27%) were new smear-positive cases under directly observed treatment standardized short-course chemotherapy (DOTS) [1].

In addition, TB is a sentinel disease for acquired immunodeficiency syndrome (AIDS) because, in contrast to most opportunistic infections, it is frequently the first indicator of human immunodeficiency virus (HIV) infection [2,3]. The risk of reactivation TB in HIV-positive cases is about 8% a year, in contrast to the 10% lifetime risk for positive tuberculin skin tests in healthy individuals [3-5].

The prevalence of resistance to anti-TB drugs shows a marked geographic difference and has important implications for the selection of an appropriate initial treatment regimen [6]. In 1996-1999, the WHO and the International Union Against Tuberculosis and Lung Disease (IUATLD) examined a total of 68,104 TB cases from various locations for drug resistance. The prevalence of resistance to at least 1 drug ranged from 2.9% in New Caledonia to 40.8% in Estonia (median, 11.1%) and that of multiple drug-resistant TB (MDR-TB) ranged from 0% in Finland and New Caledonia to 18.1% in Estonia (median, 1.8%) [6,7]. In eastern and northern Taiwan, the prevalence of resistance to any anti-TB drugs ranged from 12.3 to 35.5% and the prevalence of MDR-TB ranged from 1.2 to 17.3% [8-13]. In patients with HIV infection, TB caused by MDR bacilli is associated with widely disseminated disease. Poor treatment response is associated with an inability to eradicate the organism, and substantial mortality [14].

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AIDS associated with TB has recently become a serious public health problem, and there have been nosocomial outbreaks [15-17]. The rapid detection of resistance is critical to effective patient management.

In 1947, the TB mortality rate per 100,000 population in Taiwan was 294.44 [18]. By 1985 it had declined to 10.72 per 100,000 people, and was no longer in the top ten of the annual mortality list [18]. Of the 13,910 patients (62.7 per 100,000 population) with newly diagnosed TB in the year 2000, 1534 have since died of TB (6.91 per 100,000 population). This disease now ranks twelfth on the mortality list, but for males, it ranks tenth. In the year 2000, 137 people (10.47 per 100,000 population) in Changhua County died of TB. Only Taitung County (24.74 per 100,000 population), Hualien County (17.2 per 100,000 population), and Pingtung County (14.64 per 100,000 population) had higher mortality rates [18]. Changhua Christian Hospital did not begin to perform mycobacterial culture or antimycobacterial susceptibility testing (AST) on all suspected patients until June of 2001. Prior to this, there were no reported data on AST and HIV from central Taiwan. Here we present an analysis of the first year for which this data became available.

Materials and Methods

Patients

Changhua Christian Hospital is a 1700-bed teaching hospital, providing acute and supportive care services for patients in central Taiwan. The present study analyzed the records of all patients from whom cultures of *Mycobacterium tuberculosis* complex (MTBC) were isolated between July 2001 and June 2002. Data collected included demographic characteristics (such as age, gender, and residence zip code) and clinical characteristics (such as history of previous treatment for TB and HIV antibody titers). When multiple isolates were obtained from a patient, only the first isolate was included in this study.

Laboratory procedures

Clinical specimens were digested and decontaminated twice by using *N*-acetyl-L-cysteine (NALC) plus 5% oxalic acid and NALC plus 2% sodium hydroxide. The processed specimens were inoculated into a Mycobacteria Growth Indicator Tube and placed into the BACTEC™ MGIT™ 960 System (Becton Dickinson and Company, Sparks, MD, USA) for continuous monitoring either until they tested positive, or until the end of the testing period. An instrument-positive sample was determined by the system and confirmed by an acid-fast smear. The BDProbeTec™ ET *Mycobacterium tuberculosis* complex Culture Identification Reagent Pack was used for the identification of MTBC isolated from culture. The BACTEC™ MGIT™ 960 SIRE kit was used for AST of MTBC. Critical concentrations of isoniazid (INH, 0.1 μg/mL), rifampin (RIF, 1.0 μg/mL), ethambutol (EMB, 5.0 μg/mL), and streptomycin (SM, 1.0 μg/mL) were tested. Isolates resistant at critical concentrations were not tested for INH (0.4 μg/mL) or SM (4 μg/mL). Mono-resistance to EMB was not retested with an alternate method at either critical or higher concentration.

Primary resistance was defined as the presence of drug resistance to 1 or more anti-TB drugs in a TB patient who had never received prior treatment. Acquired resistance was defined as resistance to 1 or more anti-TB drugs in a TB patient who had a history of previous treatment. The overall resistance rate was defined as the percentage of total isolates showing resistance to any anti-TB drug. The proportion of resistance to an individual drug was calculated for those isolates that were resistant to at least that particular drug. MDR-TB was defined as an isolate that was resistant to at least INH and RIF. Data on history of anti-TB treatment and HIV antibody status were gathered retrospectively from medical records. To compare the drug resistance rates for different age groups between those with and without previous treatment, chi-squared or Fisher's exact test were performed using Epi Info 2000, Version 1.1.2.

Results

During the study period, the 7892 specimens submitted for mycobacterial cultures from inpatient and outpatient services yielded 974 isolates (12.4%) of MTBC. The contamination rate was 1.9% (151 out of 7892 specimens). 513 isolates from 348 male and 165 female patients were available for AST. The mean age ± standard deviation among males was 66.1 ± 15.6 years (range, 16 to 99 years) and among females 63.4 ± 19.2 years (range, 9 to 99 years). Sixty one percent of the patients were over 64 years old. 398 isolates (77.6%) were found to be susceptible to all drugs tested. Table 1 shows the proportion of patients harboring TB strains resistant to 1 or more anti-TB drugs according to their history of treatment. The overall resistance rate was 22.4%. The resistance rate to 1 drug was 11.5%, to 2 drugs 7.4%, to 3 drugs 2.3%, and to 4 drugs 1.2%.

Table 1. Prevalence of drug resistance to first-line anti-tuberculosis agents

Drugs	No. of resistant isolates (%)		*p*	Odds ratio (95% CI)
	Without previous TB therapy (n = 456)	With previous TB therapy (n = 57)		
INH	68 (14.9)	18 (31.6)	0.0014	2.63 (1.33-5.03)
RIF	15 (3.3)	10 (17.5)	<0.001	6.26 (2.36-15.78)
EMB	12 (2.6)	9 (15.8)	<0.001	6.94 (2.43-18.89)
SM	52 (11.4)	11 (19.3)	0.086	1.86 (0.81-3.93)
INH + RIF	11 (2.4)	9 (15.8)	<0.001	7.59 (2.62-21.15)
Any drug	94 (20.6)	21 (36.8)	0.0056	2.25 (1.18-4.16)

Abbreviations: TB = tuberculosis; INH = isoniazid; RIF = rifampin; EMB = ethambutol; SM = streptomycin; CI = confidence interval

Individual drug resistance was as follows: 86 strains (16.8%) were resistant to INH, 25 (4.9%) to RIF, 21 (4.1%) to EMB, and 63 (12.3%) to SM, respectively. There were 20 (3.9%) isolates resistant to at least INH and RIF. With the exception of SM, there were significantly higher resistance rates to INH, RIF and EMB, and significantly higher rates of MDR-TB in patients with previous treatment history.

Overall, 23.9% of isolates from male patients and 19.4% of isolates from female patients were resistant to 1 or more anti-TB drugs. Sixty three (20.9%) out of 301 male patients and 31 (20%) out of 155 female patients had primary treatment-resistant tuberculous diseases. Twenty (42.6%) out of 47 male patients and 1 (10%) out of 10 female patients had acquired treatment-resistant tuberculous diseases. A history of previous therapy with anti-TB drugs was strongly associated with an increased risk of resistance in males (42.6% vs 20.9%, $p<0.005$) and in all patients (36.8% vs 20.6%, $p=0.0056$). However, this increased risk was not significant (10% vs 20%, $p=0.68$) in females.

Table 2 shows the age and gender distribution of patients with drug-resistant MTBC infection. Isolates from female patients less than 35 years of age had the highest resistance rate compared with the entire study group (52.6% vs 21.3%, $p<0.005$). Although most strains (95.9%) were isolated from respiratory specimens, there were no differences in resistance rates either between pulmonary isolates (22.2%) and extra-pulmonary isolates (28.6%) [$p=0.49$], or between different residential locations. Only 22 patients (4.3%) had been tested for HIV antibody within 6 months of diagnosis of TB and all of the results were negative.

Discussion

The overall resistance rate in this study (22.4%) is similar to that of a study by Lee et al [11] (28.6%) in eastern Taiwan, but lower than those of Wang and Lin [10] (34%) and of Chiang et al [13] (35.5%) in studies from northern Taiwan. With respect to the primary resistance rate, our findings (20.6%) were similar to those of Lee et al [11] (16.8%), Chiang et al [13] (16.1%), and of Wang and Lin [10] (22%), but significantly lower than that in a study by Wu [19] (30.8%) 30 years ago. The overall resistance rate to INH in this study (16.8%) was similar to previous studies from Taiwan during the last 10 years (13.9-14.7%) [9,10], but lower than from a study 3 decades ago (22.6%) [19]. Wang and Lin reported a steady decrease in the resistance rate of INH from

Table 2. Age and gender distribution of patients with drug-resistant *Mycobacterium tuberculosis* complex

Pattern of drug resistance	Age (years)			
	<35 Male/female[a] (n = 16)/(n = 19)	35-49 Male/female (n = 37)/(n = 18)	50-64 Male/female (n = 83)/(n = 27)	>64 Male/female (n = 212)/(n = 101)
Single drug	0/5	5/2	6/2	30/9
Two drugs	3/4	3/0	6/1	17/4
Three drugs	0/1	4/0	1/0	3/3
Four drugs	0/0	2/0	3/1	0/0
Total no.	3/10	14/2	16/4	50/16
Percent resistant	37.1	29.1	18.2	21.1

[a]$p=0.0013$ for comparison with the other study groups by chi-squared test; odds ratio = 4.12; 95% confidence interval = 1.46-11.73.

22.1% in 1996 to 13.1% in 1999 [10]. Whether this decreasing trend in the INH resistance rate has continued deserves further investigation. The resistance rates to RIF (4.9%) and to EMB (4.1%) in this study were also significantly lower than those to RIF (10.6-18.2%) and to EMB (8.2%-12.7%) in previous reports from eastern and northern Taiwan [9-11,13]. By contrast, the resistance rate (12.3%) to SM in this study was similar to previous studies (8.3-15.4%) from Taiwan [9-11,13, 19]. The proportions of MDR-TB were higher in eastern and northern Taiwan (7.5-17.3%) than in central Taiwan (3.9%) [8-11,13]. This could be attributable to the inclusion of patients from tertiary care and referral centers for pulmonary TB in Taiwan. Only a few patients with TB were referred to our hospital for further management, and individual patients with drug-resistant TB were referred to a specialized TB center in Taichung. Under these conditions, our data may be more representative of the actual proportion of drug resistance and MDR-TB rate in the general population. However, the problem of drug-resistant TB strains in Taiwan is more serious than in many other countries [7].

As expected, the proportion of drug-resistant MTBC was higher in patients who had received prior therapy, as has been previously reported from Taiwan [9,10]. However, in a study from New York City, Salomon et al reported that prior therapy for TB was not associated with a higher risk of MDR-TB and that 15 (83%) of 18 patients were infected with multidrug-resistant isolates during their first episode of TB [20]. Yu et al also found that the MDR-TB rate was highest among patients aged 20 to 34 years and lowest among those aged 65 years or older [12]. In the present study, young female patients (<35 years of age) had the highest resistance rate (52.6%) and were at 4.12 times greater risk of acquiring resistant strains than the other groups. The reason for this needs further investigation. It is possible that some patients may have decided not to disclose prior treatment for many different reasons, or they may have acquired resistant strains during their first TB infection [6,20].

The contamination rate (1.9%) was relatively low in the present study. This could have been due to the performance of digestion and decontamination procedures twice. Due to concerns about over decontamination, we did not perform repeat procedures after April 2003. The contamination rates have remained at about 8% since then (data not shown). The resistance rate to SM was not significantly related to treatment history in our study. This could be due to a cluster of 17 (27%) out of 63 SM-resistant strains identified in March 2002. Bemer et al reported discrepant results for SM resistance in 19 strains (17.3%) using the BACTEC MGIT 960 and susceptibility testing using the BACTEC 460 TB [21]. After resolution of the discrepant results by the reference proportion method, there was a 7.3% (8 out of 110 tests) false-resistant rate and no false-susceptible results. The sensitivity of BACTEC MGIT 960 system was 100% for SM (1.0 μg/mL), and specificity was 90.5% [21]. The positive predictive value was 76.5%, and the negative predictive value 100%. Because the present study was retrospective, and since SM-resistant strains were not rechecked by the reference proportion method or at higher concentration (4.0 μg/mL), we cannot rule out the possibility of an over-estimated resistance rate to SM.

In a comparison of HIV seropositive and seronegative patients from the USA, Theuer et al found no significant differences in the clinical features of TB, such as site of disease lesion, tuberculin reaction, and chest radiographic findings [2]. Also, because it can destroy the immune system, HIV has emerged as the most important risk factor for progression of dormant TB infection in clinical disease [22]. The observed HIV prevalence rates in TB clinics vary geographically. In a previous study of patients from TB clinics in the USA, a total of 3077 specimens from consecutive patients were tested for HIV after patient identifiers were removed. The median seroprevalence rate was 3.4% (range, 0-46.3%). The highest rate was from New York City (46.3%), followed by Newark, New Jersey (34%), Boston (27%), Miami (24%), and Baltimore (13%) [23].

However, the prevalence of HIV infection among patients with active TB in Taiwan may be increasing. Wang and Lin reported that only 1 of 453 patients with TB was HIV-seropositive in 1996-1999 [10]. Chiang et al also reported that only 1 of 378 patients with active pulmonary TB was HIV-seropositive and concluded that the impact of HIV infection on the epidemic of TB in Taiwan was not significant in 1997 [24]. However, in 2000, Fang et al [25] reported that 47 (17.3%) out of 272 Taiwanese HIV/AIDS patients had pulmonary TB, which was diagnosed second only to *Pneumocystis carinii* pneumonia (24.3%) among pulmonary infections in these patients. Of the 47 patients, 20 (42.6%) had open pulmonary TB. They also found that HIV-infected patients who were immigrants from Southeast Asia (39%) had a higher risk of developing pulmonary TB than non-immigrants (15%). HIV infection is an emerging infectious disease problem in Taiwan. By December 2002, 4757 cumulative cases

of HIV infection had been reported to the Department of Health and of them, 2565 (53.9%) were newly diagnosed HIV infection within the last 3 years [26]. Although all the 22 patients in this series who were tested for HIV had negative results, most of the patients were not tested. Physicians should be aware of the serious consequence of the synergistic effects of HIV/TB coinfection. It is important that anyone with TB or tuberculous infection undergo further assessment for HIV infection or HIV antibody, because the medical management of tuberculous infection must be altered in the presence of HIV infection [2,23,27].

Over the last 2 decades, the annual rate of tuberculous infection has remained steady at between 0.35% and 0.77%. But in Taiwan's aboriginal areas the rate was 2.7 times higher than in non-aboriginal areas [18,28]. The incidence and mortality rate of TB were also higher in aboriginal areas. Although the DOTS strategy has been practiced in aboriginal areas in Taiwan since 1997, only 4.3% of the total national TB cases were treated by DOTS [18]. Only 40% of patients were detected as smear-positive TB cases, and of them, only 46.83% completed the treatment within 1 year [18]. This is far below the goal of the WHO to successfully treat 85% of detected smear-positive TB cases and to detect 70% of all such cases [6]. The WHO recommends that DOTS strategy should be implemented everywhere. Surveillance of drug resistance should not only be continued but extended [6].

In conclusion, resistance to anti-TB drugs remains frequent in Taiwan and is associated with a history of TB treatment, which may contribute to an increased risk of multiple drug resistance. The resistance rates for INH and SM were similar to previous studies from Taiwan, but the rates for RIF and EMB were lower. The rate of MDR-TB was also lower than in previous studies from Taiwan. Only a few patients were tested for HIV antibodies. Physicians in Taiwan should be more alert to the synergistic effects of TB and HIV coinfection.

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