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Resistance to first- and second-line antituberculosis drugs in Southern Taiwan: Implications for empirical treatment

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Abstract *Background:* Multidrug-resistant and extensively drug-resistant tuberculosis infections cause public health concerns worldwide. Local epidemiologic data about the drug resistance of *Mycobacterium tuberculosis* isolate (Mtb) is critical to guide appropriate empirical therapy to cure patients and restrain the spread of tuberculosis.
Methods: Antituberculosis susceptibility testing was performed for 287 Mtbs, including 63 MDR-Mtbs collected in southern Taiwan from 2011 to 2015. Tuberculosis patients were classified into newly diagnosed cases and previously treated cases based on patients' medical history.
Results: Almost no resistance was found to the tested second-line antituberculosis drugs in non-MDR-Mtbs. Higher resistance rates to ethambutol, ofloxacin, and streptomycin were observed in MDR-Mtbs compared to non-MDR-Mtbs. Among 63 MDR-Mtbs, 61.9% of patients were newly diagnosed and 38.1% were previously treated cases. For MDR-Mtb, the drug-resistance rates in previously treated cases were significantly higher for ethambutol, pyrazinamide, ofloxacin, moxifloxacin, streptomycin, and *p*-aminosalicylic acid. When MDR-Mtbs are identified in previously treated cases, empirical administration of ethambutol, pyrazinamide, ofloxacin, or moxifloxacin may not provide effective treatment. The resistance rates to these drugs were all more than 50%. Furthermore, 25% of MDR-Mtbs from previously treated patients were resistant to *p*-aminosalicylic acid.
Conclusion: We observed almost no resistance to the tested second-line antituberculosis drugs among non-MDR-Mtbs. Anti-tuberculosis regimen with pyrazinamide, ethambutol, fluoroquinolone, kanamycin, cycloserine and p-aminosalicylic acid can be empirically used for newly

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diagnosed MDR-TB cases. For previously treated MDR-TB patients, empirical ethambutol, pyrazinamide, ofloxacin, or moxifloxacin may not provide effective treatment because the resistance rates to these drugs were all >50%.

Introduction

The emergence of drug-resistant *Mycobacterium tuberculosis* (Mtb), particularly multidrug-resistant *M. tuberculosis* (MDR-Mtb) that is resistant to at least isoniazid (INH) and rifampin (RIF), has caused concerns about treating tuberculosis patients and halting the spread of tuberculosis in the community.[1–6] The World Health Organization (WHO) estimated that there are 9.6 million new tuberculosis (TB) cases and 480,000 new cases of multidrug-resistant tuberculosis worldwide in 2015. Most cases occurred in Asia and Africa.[7] There were an estimated 1.4 million TB deaths in 2015.[8] Globally, in 2014, 3.3% of new TB cases and 20% of previously treated cases had MDR-Mtbs infections.[8] In Taiwan, there were 1% of new TB cases and 6% of previously treated TB cases infected with MDR-Mtbs in 2015.[9,10] Unfortunately, only 17% of MDR-TB patients received appropriate antituberculosis treatment because of insufficient drug susceptibility testing, the cost of treatment, and the unavailability of antituberculosis drugs.[11,12] Only 48% of treated MDR-TB patients achieved a successful treatment outcome.[13] Furthermore, in 2006 the WHO defined extensively drug-resistant tuberculosis (XDR-Mtb) as a more dangerous strain of *M. tuberculosis* that is resistant to isoniazid, rifampin, and at least one second-line injectable drug (amikacin, kanamycin, or capreomycin) and one fluoroquinolone.[14,15] XDR-TB has now been reported in 105 countries worldwide, and, on average, 9.7% of patients with MDR-TB also present with XDR-TB.[13] The treatment for XDR-TB is more complicated and requires the availability of second-line antituberculosis drugs and the related antimicrobial susceptibility testing.[16]

In Taiwan, the current disease burden of tuberculosis infections totals 11,326 tuberculosis cases (48.4 cases per 100,000 population), and 591 tuberculosis-related deaths (2.54 cases per 100,000 population) occurred in 2015.[9,17] The tuberculosis incidence rate in Taiwan is decreasing when compared to the rate in 2011.[18] Among newly diagnosed tuberculosis cases, the rates of MDR-Mtbs are 0.9% in 2011 and 1.1% in 2012.[19–21]

Empirical treatment for tuberculosis is commonly practiced before resistance-testing results are available. The advantages of treatment after the results are known include reduced morbidity, mortality, and transmission.[22] Knowing the susceptibility patterns of local tuberculosis isolates to antituberculosis drugs is greatly useful for empirical treatment, especially for cases of MDR-TB and XDR-TB and those tuberculosis patients who are intolerant of or allergic to first-line antituberculosis drugs. In Taiwan, the susceptibility data regarding second-line antituberculosis drugs for isolates that are susceptible to both isoniazid and rifampin are limited.[23] In this study, we aimed to determine resistance patterns to first- and second-line antituberculosis drugs for treating tuberculosis in southern Taiwan. Analysis of resistance patterns among newly diagnosed tuberculosis patients and previously treated MDR and XDR cases may provide guidance for better empirical therapy in southern Taiwan.

Materials and methods

M. tuberculosis isolates

The Mtb isolates in this study came from the following two collections. 1. A total of 237 isolates were consecutively collected from Kaohsiung Medical University Hospital, Municipal Hsiao-Kang Hospital, and Kaohsiung Municipal Ta-Tung Hospital in Kaohsiung City, Taiwan from March to October 2015. Only one isolate was collected from each individual. Thirteen samples (5.5%) were defined as MDR-Mtbs. 2. In order to assess a larger number of MDR-Mtbs in this study, we included data regarding 50 MDR-Mtbs collected from five hospitals in Pingtung County, Taiwan, in 2011—2015. These isolates were sent to the contracted tuberculosis laboratories of Taiwan's Centers for Disease Control for drug susceptibility testing. Both Kaohsiung City and Pingtung County are located in southern Taiwan. Overall, 287 isolates were evaluated, including 224 non-MDR-Mtbs and 63 MDR-Mtbs.

Data collection

This project was approved by the Institutional Review Board of Kaohsiung Medical University (KMUH-IRB-20140243). Tuberculosis patients were classified into newly diagnosed and previously treated groups based on available medical documents and the patients' self-reports. The definitions of new and previously treated cases were based on the WHO guidelines.[6]

Susceptibility testing

The anti-tuberculosis drug susceptibility testing was performed according to the methods reported from Clinical and Laboratory Standards Institute.[24,25] The agar proportion method is used for mycobacterial susceptibility testing. Mtb suspension was inoculated onto Middlebrook 7H10 agar that contained anti-Tb drugs; agar that did not contain any drug was also used for control. The concentrations of the antituberculosis drugs used for susceptibility testing were: 0.2 μg/mL for isoniazid, 1.0 μg/mL for

rifampin, 5.0 μg/mL for ethambutol, 0.5 μg/mL for rifabutin, 2.0 μg/mL for ofloxacin, 1.0 μg/mL for levofloxacin, 0.5 μg/mL for moxifloxacin, and 1.0 μg/mL for gatifloxacin, 2.0 μg/mL for streptomycin, 5.0 μg/mL for kanamycin, 6.0 μg/mL for amikacin, 6 μg/mL for capreomycin, 5.0 μg/mL ethionamide, 430 μg/mL for cycloserine, and 2.0 μg/mL of *p*-aminosalicylic acid. Only isoniazid, rifampin, ethambutol, ofloxacin, and four injectable antituberculosis drugs (streptomycin, kanamycin, amikacin and capreomycin) were used to perform the drug susceptibility test in the clinical laboratory. Pyrazinamide DST was performed using a BACTEC™ MGIT™ 960 system (Becton, Dickinson and Company, Spark, MD, USA) as recommended by the manufacturer. The critical concentration of PZA is 100 μg/ml. DST for cycloserine has been validated on Löwenstein-Jensen media with 30 μg/mL as critical concentration.[26] If the Mtb was found to be resistant to isoniazid and rifampin, more antituberculosis drugs—including pyrazinamide, rifabutin, levofloxacin, moxifloxacin, gatifloxacin, ethionamide, cycloserine, and *p*-aminosalicylic acid—were then used to test the susceptibility. The growth of colonies in the drug-containing plate was compared to that of controls as a proportion. If the bacterial growth on the medium with the specific drug was >1% compared to the control, the strain was declared resistant to the specific drug; the strain was defined as sensitive to the specific drug when the growth rate was <1% compared to the control.

Data analysis

The qualitative variables were calculated with the chi-square test. The data were analyzed with SPSS version 10.0 for Mac (SPSS Inc., Chicago, IL, USA). Statistical significance was evaluated by a two-tailed Fisher exact test, and a *p*-value of <0.05 was considered to be statistically significant.

Results

Among the 287 isolates, 63 were MDR-Mtbs and 224 were non-MDR-Mtbs. The differences of drug-resistance patterns between non-MDR-Mtbs and MDR-Mtbs are shown in Table 1. Higher percentages of MDR-Mtbs were more resistant to ethambutol, ofloxacin, and streptomycin antibiotics than were non-MDR-Mtbs. Non-MDR-Mtbs showed almost no resistance to kanamycin, amikacin, capreomycin, or ofloxacin, except that 4% of non-MDR-Mtbs were resistant to streptomycin. Among MDR-Mtbs, 14.3% were susceptible to rifabutin. A high percentage of MDR-Mtbs (41.3%) was resistant to streptomycin, but only < 9% of them were resistant to kanamycin, amikacin, or capreomycin. Among MDR-Mtbs, 30.2% were resistant to ofloxacin, but very few (0.4%) of non-MDR-Mtbs were resistant to ofloxacin.

Four (6.3%) of 63 MDR-Mtbs were XDR-Mtbs. Among MDR-Mtb isolates, Table 2 shows the difference of drug-resistance patterns between non-XDR-Mtb and XDR-Mtb isolates. XDR-TB-Mtbs had significantly higher drug-resistance rates toward pyrazinamide, levofloxacin, moxifloxacin, kanamycin, amikacin, capreomycin, and cycloserine than did non-XDR-TB-Mtbs. However, the lowest resistance rate for XDR-TB isolates occurred with

Table 1 Comparison of drug-resistance percentages of non-MDR-Mtb and MDR-Mtb in this study.

Drugs	Non-MDR-Mtb, n = 224 (%)	MDR-Mtb, n = 63 (%)	*p*-value
First-line oral antituberculosis drugs			
Isoniazid	12 (5.4)	63 (100)	<0.001
Rifampin	1 (0.4)	63 (100)	<0.001
Ethambutol	2 (0.9)	28 (44.5)	<0.001
Pyrazinamide[a]	NT	19 (30.2)	
Rifabutin	NT	54 (85.7)	
Fluoroquinolones			
Ofloxacin	1 (0.4)	18 (28.6)	<0.001
Levofloxacin	NT	7 (11.1)	
Moxifloxacin[a]	NT	17 (27.0)	
Gatifloxacin[a]	NT	9 (14.3)	
Injectable antituberculosis drugs			
Streptomycin	9 (4.0)	26 (41.3)	<0.001
Kanamycin	0 (0.0)	5 (7.9)	<0.001
Amikacin	0 (0.0)	6 (8.5)	<0.001
Capreomycin	0 (0.0)	5 (7.9)	<0.001
Other second-line antituberculosis drugs			
Ethionamide	NT	19 (30.2)	
Cycloserine	NT	1 (1.8)	
p-Aminosalicylic acid	NT	6 (9.5)	

[a] In the MDR-Mtb group, pyrazinamide, moxifloxacin, and gatifloxacin sensitivity testing were performed in 55 cases.

Table 2 Comparison of drug resistance between non-XDR-Mtbs and XDR-Mtbs among MDR isolates in the study.

Drugs	Non-XDR-Mtb, n = 59 (%)	XDR-Mtb, n = 4 (%)	*p*-value
First-line oral antituberculosis drugs			
Isoniazid	59 (100)	4 (100)	
Rifampin	59 (100)	4 (100)	
Ethambutol	26 (44.1)	2 (50.0)	0.817
Pyrazinamide[a]	15 (28.8)	4 (100)	0.004
Rifabutin	50 (84.7)	4 (100)	0.399
Fluoroquinolones			
Ofloxacin	17 (29.3)	1 (25.0)	0.854
Levofloxacin	5 (13.2)	2 (50.0)	0.006
Moxifloxacin[a]	14 (27.5)	3 (75.0)	0.048
Gatifloxacin[a]	7 (13.7)	2 (50.0)	0.059
Injectable antituberculosis drugs			
Streptomycin	23 (39.0)	3 (75.0)	0.157
Kanamycin	3 (5.1)	2 (50.0)	0.001
Amikacin	3 (5.1)	3 (75.0)	<0.001
Capreomycin	2 (3.4)	3 (75.0)	<0.001
Other second-line antituberculosis drugs			
Ethionamide	17 (28.8)	2 (50.0)	0.372
Cycloserine	0 (0.0)	1 (25.0)	<0.001
p-Aminosalicylic acid	6 (10.2)	0 (0.0)	0.503

[a] In the non-XDR-Mtb group, pyrazinamide, moxifloxacin, and gatifloxacin sensitivity testing were performed in 55 cases.

cycloserine. The resistance rate of non-XDR MDR isolates to ofloxacin was 29.3%, but that of XDR isolates was 25.0%. We observed differences in resistance rates for four fluoroquinolones: Among XDR isolates, the lowest resistance rate was for ofloxacin (25.0%), followed by levofloxacin (50.0%), gatifloxacin (50.0%), and moxifloxacin (75.0%). In non-XDR MDR isolates, 39% were resistant to streptomycin, but the resistance rates to other injectable drugs (kanamycin, amikacin, and capreomycin) were all <10%.

Among 63 MDR-TB patients, 61.9% were newly diagnosed cases and 38.1% were previously treated cases. Table 3 summarizes the detailed drug-resistance patterns of newly diagnosed cases and previously treated cases. Among all MDR-Mtbs, the differences of drug-resistance rates in isolates from newly diagnosed cases and previously treated cases were statistically significant for ethambutol, pyrazinamide, ofloxacin, moxifloxacin, streptomycin, and *p*-aminosalicylic acid. The previously treated cases had higher resistance rates to these drugs. Among four XDR-TB cases, three were newly diagnosed and one was previously treated. No significant differences were observed in the resistance rates between isolates from newly diagnosed cases and previously treated cases among XDR-TB patients, except for ofloxacin. However, the number of XDR-Mtbs was small (data not showed).

Among the 237 Mtbs those were consecutively collected from Kaohsiung Medical University Hospital, 222 were from newly diagnosed cases and 15 were from previously treated cases. The drug resistance rates for isoniazid, rifampin, ethambutol and streptomycin were all higher for Mtbs from previously treated cases than those from newly diagnosed cases, but with no statistical significance. Only ofloxacin drug resistance rate of previously treated patient is significantly higher than the rate of newly diagnosed patients (Table 4).

Table 3 Resistance rates to antituberculosis drugs according to prior tuberculosis treatment history in MDR-TB patients.

Drugs	MDR-TB case state (%)		
	Newly diagnosed cases (n = 39)	Previously treated cases (n = 24)	*p*-value
First-line oral antituberculosis drugs			
Isoniazid	39 (100)	24 (100)	
Rifampin	39 (100)	24 (100)	
Ethambutol	12 (30.8)	15 (62.5)	0.013
Pyrazinamide[a]	6 (16.2)	13 (72.2)	<0.001
Rifabutin	36 (92.3)	18 (75.0)	0.057
Fluoroquinolones			
Ofloxacin	6 (15.4)	13 (54.2)	0.001
Levofloxacin	3 (10.0)	4 (33.3)	0.067
Moxifloxacin[a]	7 (18.9)	10 (55.6)	0.006
Gatifloxacin[a]	4 (10.8)	3 (16.7)	0.433
Injectable antituberculosis drugs			
Streptomycin	12 (30.8)	14 (58.3)	0.031
Kanamycin	3 (7.7)	2 (8.3)	0.927
Amikacin	4 (10.3)	2 (8.3)	0.801
Capreomycin	4 (10.3)	1 (4.2)	0.385
Other second-line antituberculosis drugs			
Ethionamide	11 (28.2)	8 (33.3)	0.667
Cycloserine	1 (2.7)	0 (0.0)	0.481
p-Aminosalicylic acid	0 (0.0)	6 (25.0)	0.001

[a] In the MDR-TB group, pyrazinamide, moxifloxacin, and gatifloxacin sensitivity testing were performed in 55 cases, including 37 new cases and 18 previously treated cases.

Table 4 Resistance rates to antituberculosis drugs according to prior tuberculosis treatment history in 237 consecutive tuberculosis cases.

Drugs	Consecutive Mtbs case state (%)		
	Newly diagnosed cases (222)	Previously treated cases (15)	*p*-value
First-line oral antituberculosis drugs			
Isoniazid	24 (10.8)	2 (13.3)	1.0
Rifampin	12 (5.4)	2 (13.3)	0.487
Ethambutol	6 (2.7)	2 (13.3)	0.142
Fluoroquinolones			
Ofloxacin	0 (0)	2 (13.3)	<0.001
Injectable antituberculosis drugs			
Streptomycin	14 (6.3)	2 (13.3)	0.604
Kanamycin	0 (0)	0 (0)	
Amikacin	1 (0.5)	0 (0)	1
Capreomycin	1 (0.5)	0 (0)	1

Discussion

The susceptibility patterns of non-MDR-Mtbs to second-line antituberculosis drugs is rarely reported in Taiwan.[10,27] Among patients who are not infected with MDR-Mtbs, the first-line antituberculosis drugs are effective, and at this point physicians do not necessarily require the susceptibility data for second-line drugs. Our results revealed that patients in southern Taiwan have almost no resistance to the tested second-line antituberculosis drugs (fluoroquinolones and injectable drugs) for non-MDR-Mtbs. For those with non-MDR-Mtbs infection experiencing adverse reactions to the first-line drugs, clinicians have many choices from the second-line agents.

This is the first report of MDR-Mtbs drug-resistance data for newly diagnosed and previously treated patients in Taiwan. According to published reports, a previous treatment history for tuberculosis is a risk factor for MDR-TB infection.[28,29] In this study, we found that drug-resistance rates for ethambutol, pyrazinamide, ofloxacin, moxifloxacin, streptomycin, and *p*-aminosalicylic acid in MDR-Mtbs from patients previously treated for tuberculosis were significantly higher than those from newly diagnosed MDR-TB patients. This phenomenon will influence the choice of antituberculosis drugs for MDR patients who have a history of treatment for tuberculosis. Pyrazinamide, levofloxacin, kanamycin, and cycloserine can be empirically prescribed for newly diagnosed MDR-TB cases because of the high susceptibility rates to these drugs. However, when MDR-Mtbs were identified in previously treated patients,

empirical ethambutol, pyrazinamide, ofloxacin, or moxifloxacin may not provide effective treatment because the resistance rates to these drugs were all >50%. Besides, 25% of MDR-Mtbs from previously treated patients were resistant to *p*-aminosalicylic acid. The rifamycins are long considered a mainstay of TB treatment. However, cross-resistance to rifampin and rifabutin is common, and rifampin-resistant and rifabutin-susceptible tuberculosis isolates have been reported.[30,31] There were few data about the efficacy of rifabutin in the treatment of MDR-TB. Rifabutin can be an additional drug in the treatment of MDR-TB patients with rifabutin-susceptible MDR-Mtbs.[31–34] Our result showed that 85.7% of MDR-TB patients are resistant to rifabutin. Therefore, only 14.3% of MDR-TB patients can be treated with rifabutin, which is suggested as a reasonable alternative when laboratory testings indicate susceptibility to rifabutin. Rifabutin-containing regimens can shorten the treatment duration for MDR-TB. Drug susceptibility testing for the second-line drugs is therefore very important for an effective antituberculosis therapy for MDR-TB patients, especially for those that were previously treated for TB.

Not surprisingly, XDR-Mtbs possess higher drug-resistance rates than do MDR-Mtbs for all of the antituberculosis drugs (Table 2). For XDR-Mtbs, there is no difference in drug-resistance patterns between newly diagnosed and previously treated patients, except for ofloxacin. The small sample size of XDR-TB patients is one limitation of the study and resulted in a suboptimal analysis of the differences in resistance rates between XDR-Mtbs and non-XDR MDR-Mtbs. Physicians need drug susceptibility testing results for the second-line antituberculosis drugs for each XDR-Mtbs in order to provide effective therapy for XDR-TB patients.

According to our results, streptomycin is not recommended for MDR-Mtbs treatment because of its high resistance rate, especially in previously treated tuberculosis patients or XDR-TB patients. Among the injectable antituberculosis drugs, kanamycin has the lowest resistance rate for newly diagnosed MDR-TB patients and should be considered first when the drug-susceptibility test results are not available. Ethionamide was found to have cross-resistance with isoniazid.[35] Our data showed that ethionamide does not effectively inhibit *M. tuberculosis* growth in patients with MDR-TB. Very few isolates were resistant to cycloserine, although it is bacteriostatic. Either ethionamide or *p*-aminosalicylic acid can be empirically used with cycloserine for patients with MDR-TB. However, it should be noted that 30.2% of MDR-Mtbs were resistant to ethionamide.

We compared our results in the current study with related data from southern Taiwan published in 2008.[27] Between the dates of these two studies, the percentage of XDR-Mtb among MDR-Mtb isolates decreased from 18.1% to 6.34%. The MDR-Mtbs isolated in 2008 displayed higher drug-resistance rates than those in 2016 for the following drugs: ethambutol, pyrazinamide, ofloxacin, streptomycin, kanamycin, capreomycin, cycloserine, and *p*-aminosalicylic acid. A population-based study revealed a decreasing trend of resistance to pyrazinamide, ofloxacin and amikacin, which is similar to our findings.[10] However, the susceptibility results from the consecutively collected Mtbs revealed that there were higher resistance rates to isoniazid, rifampin, ethambutol, streptomycin and ofloxacin in Mtbs from previously treated cases. It revealed resistance testing, especially for ofloxacin, was necessary for clinicians to choose appropriate treatment for previously treated TB cases.

To summarize our findings: A patient's previous treatment history is important for clinicians to know when they treat MDR-TB patients in order to prescribe effective empirical therapy. For previously treated MDR-TB patients, empirical ethambutol, pyrazinamide, ofloxacin, or moxifloxacin may not provide effective treatment because the resistance rates to these drugs were all >50%. When physicians want to use second-line antituberculosis drugs in cases with non-MDR-TB, any second-line agents can be chosen because there is almost no resistance at present in southern Taiwan. Our results suggest that fluoroquinolones or injectable anti-tuberculosis drugs can substitute for the offending agent for those with non-MDR-Mtbs infection but experiencing adverse effects to first line anti-tuberculosis agents. Anti-tuberculosis regimen with pyrazinamide, ethambutol, fluoroquinolone, kanamycin, cycloserine and p-aminosalicylic acid can be empirically used for newly diagnosed MDR-TB cases; however, drug susceptibility test for the second -line anti-tuberculosis agents is very necessary to choose effective anti-tuberculosis regimen for previously treated MDR-TB cases.

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