The Libyan International Medical University

Faculty of Basic Medical Science

Resistance to First and Second- Line Anti-tuberculosis Drugs

Monya Abdullah AL-arbi

2076

Supervised by

Dr. Huda Gargoum

Assisted by

Dr. Iftiama Naser

Report Submitted to fulfill the requirements for Scientific Research Activity

Date of Submission12/3/ 2020

Abstruct

Infections with multidrug-resistant and extensively drug-resistant tuberculosis cause public health problems around the world. Regional epidemiological data on the drug resistance of Mycobacterium tuberculosis isolate (Mtb) is crucial to direct effective clinical therapy to treat patients and to curb tuberculosis spread.
Antituberculosis susceptibility tests were conducted for 287 Mtbs including 63 MDRMtbs collected from 2011 to 2015 in southern Taiwan. Patients with tuberculosis were divided into newly diagnosed cases and previously identified based on their medical history.
Almost no resistance of non-MDR-Mtbs to tested second-line antituberculosis drugs. Anti-tuberculosis treatment with pyrazinamide, ethambutol, fluoroquinolone, kanamycin, cycloserin and p-aminosalicylic acid can be used empirically for new applications.

Introduction

Tuberculosis is a chronic granulomatous disease caused by *Mycobacterium tuberculosis* usually affects the lung but it can spread other organs or tissues. *Mycobacterium tuberculosis* is transmitted from person to person by respiratory aerosols produced by coughing.

General characteristic of *Mycobacterioum tuberculosis (Mtb)*:Shape **:**Slender, slightly curved rod, 2 - 4 µm in length and 0.2 - 0.5 µm in width, Non motile and non spore forming,Cell wall :Complex peptidoglycane arabinogalactan mycolate cell wall; 60% lipid. Stain :Poorly stained with Gram stain; neither Gram +ve nor Gram –ve, It is acid-fast because of the long chains of fatty acids called mycolic acids. Culture**:**They are aerobes,Grow slowly (15-20 hr),Optimum temperature for growing is 37˚C,Optimum pH for growing is 6.4 - 7.0, Grow only on Löwenstein Jensen media,Colonies appear in 2-6 weeks. [(2)]

The emergence of *Mycobacterium tuberculosis (Mtb*), particularly multidrug-resistant M. Tuberculosis (MDR-Mtb), which is at least resistant to isoniazid (INH) and rifampin (RIF), has caused concern in the treatment of tuberculosis patients and a halt to the spread of tuberculosis in the community.The World Health Organization (WHO) estimates that there are 9.6 million new cases of tuberculosis (TB) and 480,000 new cases of multidrug-resistant tuberculosis worldwide in 2015. Most cases occurred in Asia and Africa. In 2015, an estimated 1.4 million TB deaths were reported. Globally, in 2014, 3.3 per cent of new TB cases and 20 per cent of previously diagnosed cases were infected with MDR-Mtbs.[(1)]

Empirical tuberculosis treatment is generally performed until findings of resistance tests are visible. The benefits of treatment after the results are known include reduced morbidity, mortality, and transmission. Knowing the patterns of susceptibility of local

tuberculosis isolates to antituberculosis drugs is highly useful for empirical treatment, particularly in cases of MDR-TB and XDR-TB and those patients with tuberculosis who are intolerant to or allergic to first-line antituberculosis, The susceptibility data for second-line antituberculosis drugs for isolates susceptible to both isoniazid and rifampin are limited in Taiwan.[1]

First-Line Anti-TB Drugs

Rifampicin

is active against growing and non-growing bacilli.
The mode of action of rifampicin in *M. tuberculosis* is by binding to the β-subunit of the RNA polymerase, inhibiting the elongation of messenger RNA. The majority of rifampicin-resistant clinical isolates of *M. tuberculosis* harbor mutations in the *rpoB* gene that codes for the β-subunit of the RNA polymerase. As a result of this, conformational changes occur that decrease the affinity for the drug and results in the development of resistance.
M. tuberculosis isolates resistant to rifampicin, due to mutations in some gene it called "hot-spot region" of 81-bp spanning codons 507–533 of the *rpoB* gene. This region is also known as the rifampicin resistance-determining region.

Isoniazid

Unlike rifampicin, isoniazid is only active against metabolically-active replicating bacilli. Also known as isonicotinic acid hydrazide, isoniazid is a pro-drug that requires activation by the catalase/peroxidase enzyme KatG, encoded by the *katG* gene, to exert its effect Isoniazid acts by inhibiting the synthesis of mycolic acids through the NADH-dependent enoyl-acyl carrier protein (ACP)-reductase, encoded by *inhA*, resistance to this drug has been associated with mutations in several genes, such as *katG*, *inhA*, *ahpC*, *kasA* and NDH.

Second-Line Anti-TB Drugs

Fluoroquinolones

Fluoroquinolones are currently in use as second-line drugs in the treatment of MDR-TB. Both ciprofloxacin and ofloxacin are synthetic derivatives of the parent compound nalidixic acid, discovered as a by-product of the antimalarial chloroquine.Newer-generation quinolones such as moxifloxacin and gatifloxacin are being evaluated in clinical trials and proposed as first-line antibiotics with the purpose of shortening the length of treatment in TB .

The mode of action of fluoroquinolones is by inhibiting the topoisomerase II (DNA gyrase) and topoisomerase IV, two critical enzymes for bacterial viability. These proteins are encoded by the genes *gyrA*, *gyrB*, *parC* and *parE*, respectively .In *M. tuberculosis*, only type II topoisomerase (DNA gyrase) is present and, thus, is the only target of fluoroquinolone activity . Type II topoisomerase is a tetramer formed by two α and β subunits, coded by *gyrA* and *gyrB*, respectively, which catalyzes the

supercoiling of DNA. The main mechanism of development of fluoroquinolone resistance in *M. tuberculosis* is by chromosomal mutations in the quinolone resistance-determining region of *gyrA* or *gyrB*.[3]

Aim of Study

The purpose of this study is to identify patterns of resistance to first-and second-line antituberculosis drugs for tuberculosis care. Analysis of resistance trends among newly diagnosed patients with tuberculosis and previously treated cases of MDR and XDR may provide guidance for better clinical care in southern Taiwan.

Material and Method

In this study, the Mtb isolates came from two collections below.
Firstly: from March to October 2015 a total of 237 isolates were collected from Kaohsiung Medical University Hospital, Municipal Hsiao-Kang Hospital, and Kaohsiung Municipal Ta-Tung Hospital in Kaohsiung City, Taiwan. Of each person, only one isolate was collected. Thirteen samples were classified as MDRMtbs (5.5 per cent).
Second: In 2011-2015 we included data on 50 MDR-Mtbs collected from five hospitals in Pingtung County, Taiwan, in order to assess a greater number of MDR-Mtbs in this study. Such isolates have been sent for drug susceptibility testing to licensed tuberculosis laboratories at Taiwan's Centers for Disease Control. The city of Kaohsiung and the county of Pingtung are both located in southern Taiwan.[1]

Susceptibility testing

Anti-tuberculosis drug susceptibility testing was conducted using the methods recorded from the Clinical and Laboratory Standards Institute. For mycobacterial susceptibility testing the agar proportion procedure is used. Mtb suspension was inoculated on Middlebrook 7H10 agar containing anti-Tb drugs; agar containing no drugs was used for monitoring as well. The antituberculosis drug concentrations used for the susceptibility testing were:
0.2 mg/mL for isoniazid, 1.0 mg/mL for rifampin, 5.0 mg/mL for ethambutol, 0.5 mg/mL for rifabutin, 2.0 mg/mL for ofloxacin, 1.0 mg/mL for levofloxacin, 0.5 mg/mL for moxifloxacin, and 1.0 mg/mL for gatifloxacin, 2.0 mg/mL for streptomycin, 5.0 mg/mL for kanamycin, 6.0 mg/mL for amikacin, 6 mg/mL for capreomycin, 5.0 mg/ mL ethionamide, 430 mg/mL for cycloserine, and 2.0 mg/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. If the Mtb was found to be resistant to isoniazid and rifampin, more antituberculosis drugs dincluding pyrazinamide, rifabutin, levofloxacin, moxifloxacin, gatifloxacin, ethionamide, cycloserine, and p-aminosalicylic aciddwere then used to test the susceptibility. The growth of colonies in the drug-containing plate was compared to that of controls as aproportion. 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.[1]

Results

Of the 287 isolates, MDR-Mtbs were 63, and non-MDR-Mtbs were 224. Differences in drug-resistance trends between non-MDR-Mtbs and MDR-Mtbs: higher percentages of MDR-Mtbs were more resistant than non-MDR-Mtbs to ethambutol, ofloxacin, and streptomycin.
Non-MDR-Mtbs showed virtually no kanamycin, amikacin, capreomycin, or ofloxacin resistance except that 4 percent of non-MDR-Mtbs were streptomycin resistant. XDR-Mtbs were four out of 63 MDR-Mtbs. Among isolates at MDRMtb.The discrepancy between non-XDR-Mtb and XDR-Mtb isolates of the drug resistance trends. XDR-TB-Mtbs had significantly higher drug resistance levels than non-XDR-TB-Mtbs to pyrazinamide, levofloxacin, moxifloxacin, kanamycin, amikacin, capreomycin, and cycloserine, Cycloserine was however the lowest resistance limit for XDR-TB isolates. The non-XDR MDR isolate resistance rate to ofloxacin was 29.3%, but that of XDR isolates was 25.0%.

Discussion

In Taiwan the trends of susceptibility of non-MDR-Mtbs to second-line antituberculosis drugs are rarely reported. The first-line antituberculosis drugs are successful in patients who are not infected with MDR-Mtbs, and at this stage doctors do not necessarily require the susceptibility data for second-line drugs. Our results showed that patients in southern Taiwan have virtually no resistance to non-MDR-Mtbs tested second-line antituberculosis drugs (fluoroquinolones and injectable drugs). Clinicians have many options from second-line agents for those with non-MDR-Mtbs infection having adverse reactions to the first-line medications.

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. In this study, found that drug-resistance rates for ethambutol, pyrazinamide, ofloxacin, moxifloxacin, streptomycin, and p-aminosalicylic acid in MDRMtbs 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 have long been considered a cornerstone of care for TB. However, cross-resistance to rifampin and rifabutin is normal, Rifabutin may be an additional drug in treating MDR-TB patients with rifabutin-susceptible MDR-Mtbs. In results showed that 85.7 percent of MDR-TB patients are rifabutin-resistant. Therefore, only 14.3 per cent of patients with MDR-TB can be treated with rifabutin, which is recommended as a reasonable alternative when laboratory tests show rifabutin susceptibility. Rifabutin-containing regimens may shorten the length of MDR-TB treatment. Therefore, testing of drug susceptibility for second-line drugs is very important for successful antituberculosis therapy for patients with MDR-TB, particularly those previously treated with TB.

XDR-Mtbs have higher drug resistance levels for all antituberculosis drugs than MDR-Mtbs do. Except for ofloxacin, there is no disparity in drug-resistance trends for XDR-Mtbs between newly diagnosed and previously treated patients. The small sample size of XDR-TB patients is one drawback of the study and resulted in a suboptimal review of the variations between XDR-Mtbs and non-XDR MDR-Mtbs in the resistance levels. In order to provide effective therapy for XDR-TB patients, clinicians need drug susceptibility test results for the second-line antituberculosis drugs for each XDR-Mtbs.

Because of its high resistance rate, streptomycin is not recommended for treatment with MDR-Mtbs, particularly in previously treated patients with tuberculosis or with XDR-TB. Kanamycin has the lowest resistance rate among injectable antituberculosis drugs for newly diagnosed MDR-TB patients, and should be considered first when the drug-susceptibility test results are not available.

It was found that etionamide has cross-resistance with isoniazid. In data it was shown that ethionamide does not inhibit M effectively. Growth of tuberculosis in MDR-TB patients; Although it is bacteriostatic, very few isolates were cycloserin resistant. For patients with MDR-TB, either ethionamide or p-aminosalicylic acid may be employed empirically with cycloserine. It should be noted, however, that 30.2 per cent of MDR-Mtbs were ethionamide resistant.[(1)]

Conclusion

Empirical ethambutol, pyrazinamide, ofloxacin, or moxifloxacin may not provide effective treatment for previously treated MDR-TB patients because the resistance levels to these medicines were all > 50 percent. Anti-tuberculosis regimen with pyrazinamide, ethambutol, fluoroquinolone, kanamycin, cycloserine, and p-aminosalicylic acid may be used empirically in newly diagnosed cases of MDR-TB; however, the second-line anti-tuberculosis drug susceptibility test is very important to pick an effective anti-tuberculosis regimen in previously treated cases of MDR- TB.

References

1. Kuo C, Wang W, Huang C, Chen Y, Lu P. Resistance to first- and second-line antituberculosis drugs in Southern Taiwan: Implications for empirical treatment. Journal of *Microbiology, Immunology* and Infection. 2018;51(1):88-93.

2. Levinson, warren. Review of medical *microbiology and immunology*. 23rd ed. The mcgraw-hill companies, 2008.

3. Palomino J, Martin A. Drug Resistance Mechanisms in *Mycobacterium tuberculosis* 2020.