

**BIOCHEM118: Genomics and Medicine**  
**Final Project: Multiple Drug Resistant Tuberculosis**  
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**Background: Tuberculosis**

In 1952, Selman Waksman accepted the Nobel Prize for his discovery of streptomycin, a drug that aimed at combating tuberculosis: eliminating “The Great White Plague”. While this was a breakthrough in many respects, many were hesitant as to the efficacy of this drug. That same year, Jorgen Lehman discovered para-aminosalicylic acid. The combining these two medications provided a more effective treatment for patients, and helped minimize the generation of drug-resistant organisms.

Tuberculosis, most commonly referred to as TB, is a bacterial infection caused by a germ called *Mycobacterium tuberculosis*. The bacteria spreads through the air, transferred from the host through coughing, sneezing, and even talking. These exhalations release TB germs known as bacilli into the air. Therefore, the risk of spreading increases the longer a person is in contact with the contaminated air; if ventilation is poor; or if the victim remains untreated. It subsequently attacks the lungs causing a myriad of symptoms including the following: a bad cough that lasts 3 weeks or longer; weight loss; coughing up blood or mucus; weakness; fever and chills; or night sweats. For every three people in the world, one is affected with tuberculosis. Diagnosing active TB helps prevent new infections, which is necessary because patients with active TB will go onto infecting 10-15 other people per year until they are treated or die. TB can be fatal if not treated properly and on a timely basis. The core drugs used to treat MDR-TB are isoniazid and rifampin. With proper medication taken until completion, victims have a chance of survival. What happens, however, when the tuberculosis does not respond to the drugs? What happens when the bacteria have outsmarted the very drugs designed to destroy them?

TB can usually be treated with a series of anti-TB drugs; however if these drugs are misused multi-drug-resistant TB may emerge; if the second-line drugs are continued to be misused, XDR-TB can developed. Drug resistance is the reduction in effectiveness of a drug and illustrates the evolution in microorganisms. These microbes, which are not susceptible to the drug's effects are capable of surviving initial treatment and therefore, their drug resistant traits are selected for in future generations. This will eventually produce a population of drug resistant microbes. Many respiratory infections, HIV/AIDS, and tuberculosis have shown resistance to first line defense agents. The result is extensively drug resistant bacteria that do not respond to prescribed drugs.

### **Extensively drug-resistant tuberculosis**

Extensively drug-resistant tuberculosis is a form of the disease, whose microbes are resistant not only to isoniazid and rifampicin, but also any fluoroquinolone and at least one of three injectable second-line drugs (amikacin, capreomycin, and kanamycin). This is the most extreme form of drug-resistance. The WHO Global Task Force agreed on this highly specialized definition of XDR-TB in October of 2006. XDR-TB has developed from two main factors: first, the inadequacy of TB treatment administration by the public health infrastructure, and second, the ongoing prevalence of the HIV epidemic. XDR-TB is particularly dangerous because it may be transmitted to patients who have not been previously treated for the disease. Therefore, it is possible to spread the most severe case of TB, instantly. This rapid spread of XDR-TB is terrifying but notable, as although this disease is more prevalent in developing countries, there have been 49 cases reported in the United States between 1993 and 2006.

Drug resistance is based on the ability of bacterial TB mutants to resist chemotherapy.

There are three main mechanisms that can produce epidemics of drug-resistant diseases: first is

known as acquired resistance, where

random strains of the microbe

become drug-resistant strains;

second, is amplified resistance where

the strains become drug-resistant

because of inappropriate procedures

and chemotherapy; finally is

transmitted resistance, which

involves the transmission of drug-

resistant cases. Tuberculosis-drug

resistant strains have occurred

traditionally by acquired and

amplified drug resistance. However,

the drastic spikes in the rise of XDR-tuberculosis cases is linked to transmitted resistance.

The incidence of highly drug-resistant tuberculosis is much higher in poor regions,

among the homeless, and regions of drug abuse. For example, during the TB outbreak in San

Francisco, 200 of the 100,000 people in the Tenderloin, one of the most poor neighborhoods in

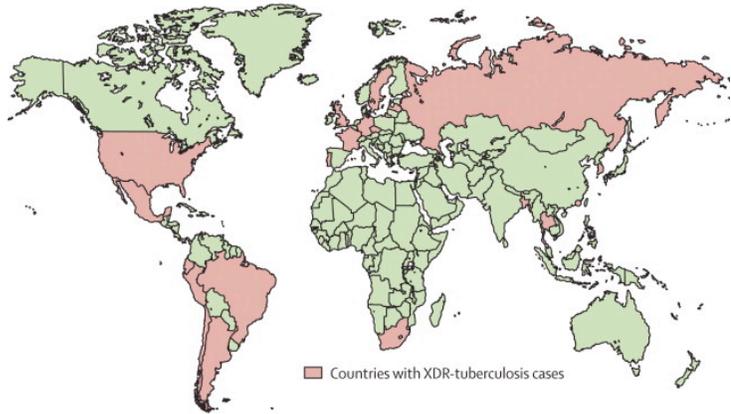
the region, were affected, an incidence higher than Sub-Saharan Africa (Brutlag). Figure 1, to the

right, illustrates the high increase and rapid spread of the XDR-tuberculosis throughout the

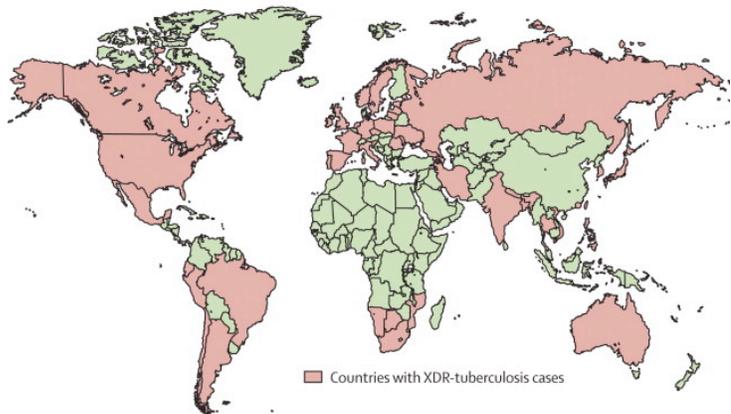
world. Without viable cures and adequate control mechanisms, this spread will definitely

continue, uncontrollably.

December, 2006



June, 2008



## **Diagnosis**

To detect TB, scientists can perform a series of tests: tuberculin skin testing, analysis of clinical signs, and sputum microscopy; new methodologies such as nucleic-acid amplification and interferon- $\gamma$  release assays are also available for use. The emergence of multi-drug resistant tuberculosis (MDRTB) means that detection of drug resistance is necessary for stopping the spread of drug-resistant strains. The microscopic observation drug-susceptibility assay is a low-cost, low-tech tool for high-performance detection of TB and MDRTB.

If TB bacteria are found in the sputum, the diagnosis of TB can be made in only a few days; however, these tests cannot predict whether or not the form of TB is drug-susceptible or drug-resistant. Most drug-susceptibility testing has been the agar proportion method; this technique requires several weeks for results to be determined. This form of testing can detect drug resistance to standard TB drugs. By focusing on the identification of rifampicin resistance and using this as a marker for other drugs, drug susceptibility can become much more efficient.

Molecular methods can also be used to detect drug-resistance, including line-probe hybridization assays in conjunction with nucleic-acid amplification, molecular beacon assay, luciferase mycobacteriophage strategy, dideoxy fingerprinting, direct sequencing of PCR products and heteroduplex analysis. There are also a series of diagnostic methodologies for the rapid identification of resistant *Mycobacterium tuberculosis* (MTB): microscopic observation drug susceptibility assay; nitrate reductase assay; phase amplification; and nucleic acid amplification. Microscopic observation drug susceptibility assay involves the microscopic visualization of MTB colonies in liquid medium containing antibiotics. Its advantages are that there is not need for “new” equipment and technical training for the procedure is simple. The disadvantages are the potential for culture contamination and the need for biosafety level 3

laboratories. MODS assay was originally developed in Peru; and costs only three dollars per test for liquid based detection of TB. This method was ideal for implementation in regions where resources were limited. MODS has three main (primary) benefits: first, MTB grows in liquid media much faster; second, MTB is detected earlier in liquid media meaning that its growth can be distinguished from atypical mycobacteria or contamination; finally, this method can detect multi-drug resistance. Drugs such as isoniazid and rifampicin can be incorporated into the MODS assay to check for resistance. Nitrate reductase assay involves color changes based on the ability of MTB to reduce nitrates to nitrites in mediums containing antibiotics. The benefit of this mechanism is can make sensitive and specific detections in resistance to isoniazid and rifampin. The only disadvantage is that it requires a solid medium. Phase amplification involves the formation of clear plaque in medium containing antibiotics due to proliferation of injected mycobacteriophage within viable MTB cells. Its advantages are that no specialized equipment is required, and the performance is generally strong. The final method for rapid identification of resistant mycobacterium tuberculosis is nucleic acid amplification, which requires the direct detection of genes associated with antibiotic resistance in samples or isolates. The advantage to this procedure is that it can recognize large number of genes; its disadvantage is a high risk of contamination.

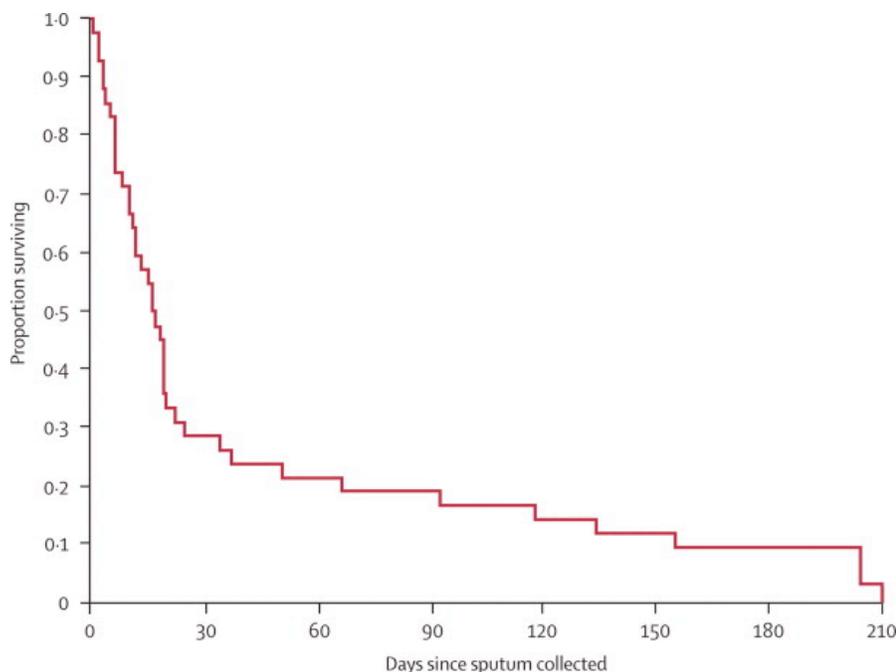
## **Treatment**

The two main goals of TB treatment are to simplify current treatment options and to find new alternatives to combat multi-drug resistant TB. Treatment for TB includes several kinds of medicine: isoniazid, also called INH, rifampin, ethambutol, and pyrazinamide. Treatment techniques for MDR-TB and XDR-TB require chemotherapy and the use of second-line drugs. These drugs tend to be much more toxic and expensive than the standard anti-TB treatment and

can cause many serious side effects such as hepatitis and depression. XDR-TB is associated with a much higher mortality rate than MDR-TB: yet, the successful treatment of both diseases depends on the extent of drug resistance, the severity of the disease, and the state of the patient's immune system (the stronger the immune system, the more chances of successful recovery).

There are various forms of treatment that can be used to help mitigate the effects of TB. Treatment with antiretroviral therapy can aid in reducing the number of immuno-compromised patients that could get TB. This therapy linked with isoniazid therapy can help enhance the effects of HIV therapy and reduce the probability of TB. New therapy options include the use of cytokines because chemotherapy is ineffective for patients with extensively drug-resistant tuberculosis. The only limitations of the use of cytokines are their high cost and toxicity. However, results from trials simply using cytokines have been ineffective which suggests that more research regarding immune response to cytokines is necessary.

*Figure 2 illustrates the survival rates after sputum collection in patients with XDR tuberculosis. The curve illustrates rapid mortality rates in a rural area in KwaZulu-Natal, South Africa.*



## **Prevention**

The Global Plan to Stop TB 2006-2015 aims to halve tuberculosis deaths by 2015, when compared to prevalence in 1990. Ministers from high M/XDR-TB burden countries plan to meet in Beijing in April of 2009 to address the threat and spread of XDR-TB. Currently, the average treatment completion rate is below 85%, meaning that many patients begin treatment and do not terminate it, allowing the microbes to develop into multi-drug resistant strains. In order to prevent the epidemic of XDR-TB, there will need to be drastic improvements in surveillance of the disease, better control, and more development of new therapeutics, aimed at early detection of drug-resistant strains. Prevention and prediction of future drug-resistant tuberculosis requires an understanding of amplification probability and cure rates. TB affects people differently and develops different strains rapidly; understanding all of the complex expressions of the disease will assist in producing plausible vaccines and other preventative measures. This can be done through additional lab studies and testing of different strains of the bacteria. More funding and the creation of better infrastructural systems for disease control are also necessary. These structures would aim at regulating testing, therapy, and treatment to ensure that patients were following through with their treatment program. Medication ceases to be effective when it is not completed as prescribed. Other preventative measures include: getting tested regularly if you are at a high risk of TB (people with HIV or from regions with high rates of TB); and keeping the immune system healthy by eating lots of fruits and vegetables.

## **Vaccinations**

In the 1870s, Louis Pasteur discovered that tiny microbes caused these diseases and that they could be stopped through preventative vaccinations. These vaccinations are necessary because without them the chances of spreading an infectious disease increase exponentially. However,

many families choose not to vaccinate their children either from lack of access to vaccines, religion, or fear of risks. The importance of vaccination is demonstrated through the notion of herd immunity: herd immunity proposes that in diseases passed from one person to another, it is more difficult to get an infection when large numbers of the population are immune. By vaccinating more of the world population, we can substantially decrease the number of people who are at risk of infection. BCG is a vaccine that is prepared from a strain of live bovine tuberculosis bacillus that has been weakened in culture. This vaccine, which is meant to counter tuberculosis, was first used in humans in 1921 after extensive research throughout World War I. However, because of resistance, the vaccine was not widely used until after the Second World War. The BCG vaccine prevents some forms of TB in children, particularly tuberculous meningitis, but is not very effective when applied to adults with XDR-TB. The efficacy of the vaccine changes often due to genetic variation in BCG strains and genetic variation in populations. Therefore, there needs to be additional funding and more vaccines produced to combat these diseases; in the United States mass immunization of BCG has never occurred. This is because it is not very effective and causes a false-positive result on a Mantoux skin test. Since BCG does not protect 100% of the people inoculated, it would not be an effective vaccine to induce herd immunity.

### **XDR-TB and HIV/AIDS**

The incidence of XDR-TB and HIV is related; their interaction is bidirectional. Throughout southern Africa, the proportion of individuals with HIV is greater than 25% among those who are also victims of active tuberculosis. HIV, as it decreases the strength of the immune system, increases the chances of TB reactivation by 20-fold and also increases the chances of re-infection with more drug-resistant strains. The bacteria that cause TB become active when stimuli

decrease the immunity of the patient: mechanisms that can decrease the immune response include HIV, aging, and other medical conditions. Moreover, TB causes cellular activation and excessive cytokine and chemokine production. This production stimulates the replication of HIV, which thus increase the rate at which the patient will attain AIDS and severely decreases the overall rate of survival.

### **Conclusion**

The task facing scientists today is the necessary improvement of global tuberculosis control by enhancing the testing and care of HIV-infected populations. Multi-drug resistance infectious diseases has the potential to terrorize and destroy all of humanity, as it can spread rapidly, if not controlled and regulated. Accordingly, it is our moral obligation to stop the spread of XDR-TB before it is too late.

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