MODELLING THE CONTAINMENT OF MYCOBACTERIUM TUBERCULOSIS STRAIN

Abukari Alhassan, MSc.
University for Development Studies, Navrongo Campus, Ghana
Kaku Sagary Nokoe ,PhD
University of Energy and Natural Resources, Sunyani, Ghana
Kwabena Kyei Aboagye, PhD
University for Development Studies, School of Medicine, Tamale, Ghana

Abstract

Drug resistance in mycobacterium Tuberculosis (MTB) undermines the efficacy of Tuberculosis treatment in individuals and of Tuberculosis control programmes in populations. Non compliance of anti Tuberculosis drugs can result in drug resistance MTB strain. Some fluctuation tests demonstrate that mutation to resistance is 2.56×10^{-8} and 2.25×10^{-10} per bacterium per generation respectively for isonized and rifampicin. Here, we propose a model for the growth of initial drug sensitive bacilli population taking into consideration conferred mutations. This was validated against experimental data. The model shows that if the total number of the MTB strain is less than 39,062,500 and 4,444,444,444 with selective effect to isoniazid and rifampicin respectively, the explosion can eventually be contained. This is far less than clinical bacterial load of 10^{10}. This finding may also help explain the pharmacodynamic properties of the "first line" anti Tuberculosis drugs.

Keywords: Branching process modeling, disease transmission, drug susceptibility, stochastic process

1.0 Introduction

Tuberculosis has been found in the mummies of ancient Egyptians and Andean Indians demonstrating that it has been in humans for thousands of years. It was first identified by Dr. Robert Koch in 1882. The disease was historically referred to as “consumption.” The scientific name for the Tuberculosis microbe is ***Mycobacterium tuberculosis or MTB***. Beneath a microscope, it has a long rod-like shape and thick, waxy-looking coat. The Bacteria are single-celled organisms which can exist either as independent or

as parasites. The thick waxy cell wall allows the germ to spread through the air and survive on surfaces for weeks.

Unlike most bacteria which divide within minutes or hours, TB bacterium splits into two only once every 16-20 hours. This asexual process is known as binary fission. MTB has all the necessary genetic material to reproduce so it does not require a host.

According to the United States global health policy fact sheet in 2010, One-third of the world's population, or two billion people, carry the TB bacteria, more than 9 million of whom become sick each year with "active" TB which can be spread to others. "Latent TB" disease cannot be spread.TB disproportionately affects people in resource-poor settings, particularly those in Asia and Africa (WHO, 2009, 2010).TB cases and deaths occur in developing countries, constituting significant challenges to the livelihoods of individuals primarily during their most productive years with more than 90% of the new cases in developing countries (WHO, 2009).

When active TB is manifested, the infection is normally treated with antibiotics, thereby providing an external pressure for the selection of antibiotic resistant bacilli. The risk of resistance development is determined by a number of different factors, including the antibiotic selective pressure (set by the number, dosing and quality of the used drugs), any pre-existing resistances in the infecting clone, the immune status of the treated individuals and their compliance with the drug (Mariam *et al.*, 2011).

There have been some challenges in the past decades in the fight against TB. We categorized the challenges into two: First, treatment challenges in terms of how to deal with drug-to-drug interactions, over dose regimen to children and infants due to little or no data on best treatment. Secondly, challenges in terms of drug resistance which researchers have still not made great achievements on how best to treat drug resistant TB. Many high-burden settings lack the facilities to detect drug resistant TB as well as difficulty in understanding all of the mutations that correspond with resistance.

According to The World Health Organisation (WHO) almost 500,000 people develop multidrug-resistant TB (MDR-TB) every year (WHO, 2009). This form of TB is highly resistant to conventional front-line drugs and places additional force on public health care systems. Globally in 2012, an estimated 450,000 people developed MDR-TB and there were an estimated 170,000 deaths from MDR-TB (WHO, 2013). MDR-TB is defined as disease resulting from MTB infection by strains that are resistant to frontline TB drugs, isoniazid (INH) and rifampicin (RIF) (Gagneux, 2006). Treatment for drug sensitive (DS) TB is less than optimal, partly because the onerous 6 month treatment period leads to noncompliance, especially as patients start feeling better. MDR-TB treatment regimens are even longer, and the drugs

used are more expensive and have extensive side-effects with fewer than one in five MDR-TB patients being correctly diagnosed with MDR-TB (Mel, 2012).Interruption of treatment is one of the factors which allow for the development and maintenance of drug-resistant (DR) MTB strains; however, biological factors are likely to play a significant role as well (Warner & Mizrahi, 2006).

Our objective in this paper is to provide a model that will enable the understanding of the initial evolutionary process of MDR TB strains under selective pressure, as well as provide the basis for comparing and validating experimental data for better understanding.

2.0 Related Studies

There has been extensive research on the mathematical modelling of TB. For convenience of related literature, we classify them into two phases: first, modelling the transmission dynamics of TB among and within human populations, and secondly, studies involving the dynamics of MTB pathogens.

Iwasa *et al*., (2003,2004) used a multi-type branching process to model the order of movement of mutants that leads to a drug resistant mutant. Their model described different mutation networks in the appearance of a drug resistant mutant and calculated the probability of drug resistance.

Ribeiro and Bonhoeffer (2000) compared two possible scenarios for the outgrowth of drug resistant pathogens: either a drug resistant strain exists before the treatment or it appears after the treatment starts. They also used stochastic simulations to compare the two possibilities.

Colijn *et al*., (2011) used a stochastic birth-death model to estimate the probability of the emergence of multidrug resistance during the growth of a population of initially drug sensitive TB bacilli within an infected host. They found that the probability of the emergence of resistance to the two principal anti-TB drugs prior to therapy ranges from $10^{-5} - 10^{-4}$. Their model is analogous to Luria and Delbruck (1943), Lee and Coulson (1949) and Zheng (1999).

Schinazi (2006) presented a discrete time stochastic process to evaluate the risk of a treatment induced drug resistance. He assumed that in the absence of treatment, a drug resistant pathogen is outcompeted by the drug sensitive pathogen and it rapidly dies out if it appears. However, in the presence of a drug, the drug sensitive strain is weakened.

Blower *et al*., (1996) studied the development of drug resistance focusing on threshold R_o. Their study found that control programs could become perverse, though this requires a rather high probability of acquisition of drug resistance due to treatment. Dye *et al*., (1998) presented a model for drug resistant TB alone. They estimated R_o using Monte Carlo for drug

resistant TB. In their study, it was shown that evidence of short course chemotherapy can bring resistance strain under control and preventing drug resistant TB from emerging which can be achieved by meeting the World Health Organisation (WHO)'s targets for case detection and cure.

Gillespie (2002) asserted that risk of mutants emerging in TB patients depends partly on combined mutation rate of 10^{-25}/bacterium/generation. He generated the formular for computing the risk of mutation which was found out to be an oversimplification if the MTB are found in different compartments.

The motivation we derived from some of these literature is that, most of these modelling discussed the transition of MTB, risk of resistance and general behaviour of the MTB strain. This study seeks to model the containment of MTB strain using stochastic branching process, Though analagous to other studies, we focused on the containment of the MTB strain as they evolve with risk of resistance to first line anti TB drugs. We validate the model with experimental results from some fluctuation analysis**.**

3.0 The Model

We first of all present our branding process model to characterize the propagation of the MDR TB strain .

We use M to denote the total number of MTB strain and k to denote the number of drug resistant MTB strain. The probability of finding a successful drug resistant MTB strain is $p = k / M$

For first line anti TB drugs, the mutation rates to resistance to the drugs is 2.56 X 10^{-8} and 2.25 X 10^{-10} mutations per bacterium per generation respectively for (Isoniazide) INH and RIF (Rifampicin). (Canetti *et al*., 1969) and (Hsie & Bryson, 1950).

Since our objective is to model the selective effect of first line anti TB drugs on the MTB colony, our p measures how wide spread the resistance MTB strain is. In short, p is the density of the MDR MTB strain.

For the early phase of the propagation ,we assume that total number of MTB colony is not more than "M ". therefore, we put an upper bound of M on the number of times the MTB strain can multiply. We characterize what values can M take to achieve an extinction probability of 1.

3.1 Branching Process

The Galton Watson branching process models a population in which each individual in generation n independently produces some random number of individuals in generation $n+1$ according to a probability distribution that does not vary from individual to individual (Karlin *et al*.,1975).

All MTB strain can be classified into generations in the following manner. The initial MTB strain belongs to the 0^{th} generation. all MTB strain that are directly given birth to by the initial parent are the 1^{st} generation regardless of when they are given birth to. In general, an MTB strain k_b is an $(n+1)^{th}$ generation if it is given birth to directly by an MTB strain k_a from the n^{th} generation. k_b is also called an offspring of k_a. All MTB strain forms a tree if we draw a link between a parent and its offspring.

Let X be a random variable with offspring distribution $(p_0, p_1, p_2, ..., p_k)$, during the initial phase of the evolution of the MTB strain, X is a binomial random variable (M, p)

$$P(X = k) = \binom{M}{k} p^k q^{m-k} \qquad [1]$$

$q = 1 - p$ and p is the density of the MTB strain. p remains constant since the number of MDR TB strain is smaller than the drug sensitive TB strain.

Further,we let Z_n be the number of MTB strain in the n^{th} generation. Z_0 is the number of initial MTB strain.

In the early phase of the evolution of the MTB strain, each MTB strain in the n^{th} generation produces a random number of MTB strain according to the same probability distribution. These newly produced MTB strains are $(n+1)^{th}$ generation.

let $X_k^{(n)}$ denote the number of drug sensitive MTB strain that have become resistant by the k^{th} MDR MTB strain in the n^{th} generation. The number of MDR MTB strain in the $(n+1)^{th}$ generation can be expressed as:

$Z_{n+1} = \sum_{k=1}^{z_n} X_k^{(n)}$,where $X_k^{(n)}$ are independent binomial (*M* ,*p*) random variables.

During the initial MDR MTB strain evolution, each MDR MTB further produces offspring independently and according to the probability distribution in [1]. Therefore the spread of the MDR MTB strain in each generation $(Z_n, n \geq 0)$ forms a branching process.

3.1.1 Branching process and probability generating functions.

A discrete random variable *X* with probability mass function *p(x)* has a probability generating function (PGF)

$$G_X(s) = E(s^X) = \sum_{x=0}^{\infty} p(x) s^x \qquad [2]$$

If Z_n is the number of individuals at time *n* (Z_0=1), and X_i is the number of offspring of individual of *i*.

$$Z_2 = X_1 + X_2 + ... + X_{Z_1}$$

So,

$$G_2(s) = G_1[G_1(s)]$$

and for the n^{th} generation, Let Y_i= the number of offspring of i^{th} member of $(n\text{-}1)^{th}$ generation.

$$Z_n = Y_1 + Y_2 + ... + Y_{Zn-1}$$

and so

$$G_n(s) = G_{n-1}[G(s)]$$

$$G_n(s) = G_{n-2}[G[G(s)]]$$

$$G_n(s) = \vdots$$

$$G_n(s) = G[G[.....[G(s)].....] \quad [3]$$

Moments can be calculated from [2] as:

$$G(s) = \sum_{x=0}^{\infty} p(x)s^x = E(s^X)$$

Then

$$G'(s) = E(Xs^{X-1})$$

$$G'(1) = E(X)$$

Likewise

$$G''(s) = E(X(X-1)s^{X-2})$$

$$G''(1) = E(X(X-1)) = E(X^2) - E(X)$$

So

$$Var(X) = E(X^2) - E^2(X)$$

$$Var(X) = [G''(s) + E(X)] - G'(1)^2$$

$$Var(X) = G''(1) + G'(1) - G'(1)^2$$

Generally,

$$\mu = G'(1), \quad [4]$$

$$\sigma^2 = G''(1) + \mu - \mu^2 \quad [5]$$

3.1.2 Mean size of nth generation of the branching process.

Let $\mu = E(x)$ *and let* $\mu_n = E(Z_n)$. We know that $\mu = G'(1)$. From [3]

$$E(X) = G'(1)$$

$$G_n(s) = G_{n-1}[G(s)]$$

$$\Rightarrow G_n'(s) = G_{n-1}'[G(s)]G'(s)$$

$$G'(1) = G_{n-1}'[G(1)]G'(1)$$

$$= G_{n-1}'(1)G'(1)$$

So $\mu = \mu_{n-1}\mu = \mu_{n-2}\mu^2 = ... = \mu^n$

It can be seen that as $n \to \infty$

$$\mu_n = \mu^n \to \begin{cases} \infty & \mu \succ 1 \\ 1 & \mu = 1 \\ 0 & \mu \prec 1 \end{cases}$$

So at first sight it looks as if the generation size will either increase unboundedly ($\mu \succ 1$) or die out ($\mu \prec 1$).

3.1.3 Extinction Probability For The MDR TB Strain.

For mathematical convenience, we assume that the initial number of MDR MTB strain is 1.

Let $\mu = EX$ be the mean number of offspring per MDR MTB strain. Let γ denote the probability that the population of MDR MTB strain dies out eventually.

$$\gamma = P(MDR\ MTB\ strain\ dies\ out) = P(Z_n = 0\ \ for\ some\ \ n)$$

In the case of our study, the extinction probability measures the likelihood of the MDR MTB strain dying out after a number of generations.

When $\gamma = 1$, we are certain that the population of the MDR MTB strain cannot be exploded for an arbitrarily number of generations.

The proposition below provides a sufficient and necessary condition for the extinction.

Proposition:

Let p be the density of the MDR MTB strain and the total number of drug sensitive MTB be *M*. Then $\gamma = 1$ if and only if $M \le \frac{1}{P}$

Proof: Recall that the growth of the MDR MTB strain forms a branching process where each MDR MTB strain independently produces a random number of offspring.

Let X represent the random variable of the number of offspring produced by each MTB strain. Since the total number of MTB strain is M ,

then X is a binomial random variable with distribution as in [1] with mean $E(X) = Mp$.

$\gamma = 1$ if and only if $E(X) \leq 1$ (Ross, 1996)

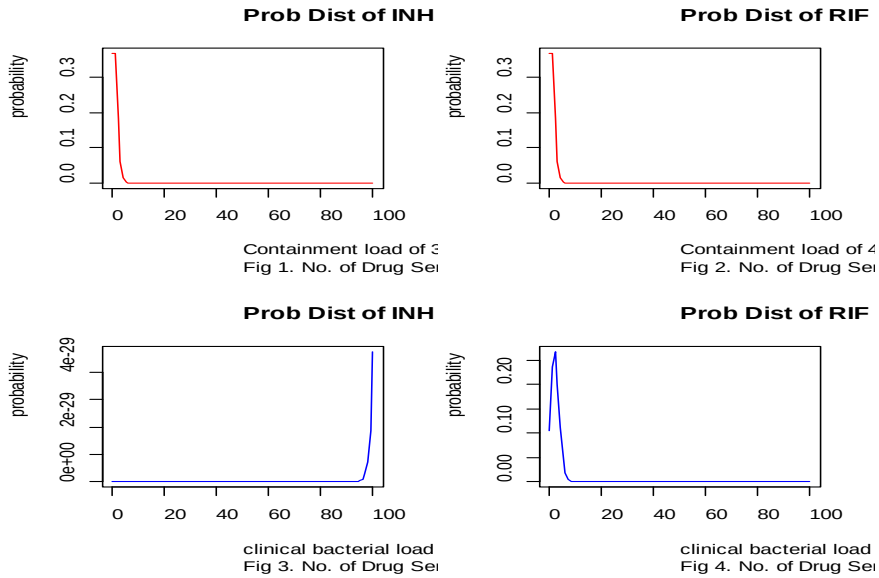
Therefore $\gamma = 1$ if and only if $M \leq \frac{1}{P}$.

The practical implication is that if we limit the bacteria colony to less than $\frac{1}{P}$, the evolutionary process of the MDR MTB strain can eventually be contained.

We use this to provide the basis for assessing fluctuation test regarding the frequency of mutations of the MTB strain.

Table 1. Analysis of Fluctuation Test.

Firts Line Anti TB Drugs	Mutation Rate Per Bacteria/Generation	1/P	Clinical Bacteria Load
INH	2.56×10^{-8}	39,062,500	10^8-10^{10}
RIF	2.25×10^{-10}	4,444,444,444	10^8-10^{10}



Figures 1 and 2 illustrate our claim that the explosion of the MTB strain can be contained with conferred resistance to INH and RIF. Figures 3 and 4 demonstrate the difference between INH and RIF's resistance as the DS strain explodes.

4.0 Results and Discussion

Drug resistant M. tuberculosis arise in clinical practice when therapy is inadequate.This could be the result of inadequate prescription or because the patient fails to adhere fully to an appropriate treatment regimen. The basis for the molecular understanding of this resistance has been established

(Musser, 1995). In this study, we have used a branching process model for the growth of an initial drug sensitive bacilli population taken into consideration conferred mutations.

The beauty of this model is that it can form the basis for comparing several fluctuation analysis. Different authors have come out with different mutation rates for the MTB strain under different susceptibility testing to understand the dynamics of MTB strain in the context of in vitro infection. Shimao (1997) and Gillespie (2002) indicated a population size of $10^8 - 10^{10}$ bacilli organisms as the bacterial load. Fluctuation test demonstrated that resistance to specific anti TB drugs arises spontaneously at a rate of $10^6 - 10^9$ cell divisions depending on the drug.

Mutation from DS to DR for both INH and RIF declined sharply with increasing DS load for our containment model in Figs 1 and 2. In Fig 3, the mutation rate continued to increase at a constant rate after the 90^{th} generation as the DS strain exploded to the clinical bacterial load. Surprisingly, In Fig 4, mutation for RIF declined sharply after the 10^{th} generation and maintained a constant rate as the DS strain hit the clinical bacterial load.Could this be due to the fact that clinical response to chemotherapy for RIF was considerably poorer in patients with initial RIF resistance?.

Using INH and RIF which are the common first line anti TB drugs, proposition 1 implies that if the total number of MTB strain is less than 39,062,500 and 4,444,444,444 respectively for INH and RIF, the explosion can eventually be contained. This is far less than the clinical bacterial load of $10^8 - 10^{10}$.

5.0 Conclusion

Clinically, the symptomatic population size of 10^{10} can be kept under control, hence, further critical concentration levels of the anti TB drugs can be studied to improve the treatment process as well as reduce the frequency of mutations.

Appropriate dosages of INH and RIF can be determined to continue treatment for the DS MTB strain as our containment model suggest a reduction in the mutation frequency with increasing DS population given the assumption of compensatory fitness cost for the DR strains

References:

Blower, S. M., Small, P.M., & Hopewell, P. C. (1996). Control strategies for Tuberculosis epidemics: New models for old problems. *Science*. 273. 497–500.

Canetti, G., Fox, W., Khomenko, A., Mahler, H.T., Menon, N.K., Mitchison, D.A., Rist, N., & Smelev, N.A., (1969). Advances in techniques of testing tycobacterial drug sensitivity, and the use of sensitivity tests in Tuberculosis control programmes. *The Bull*. 41. 21-43.

Colijn, C., Cohen T., Ganesh, A., & Murray, M. (2011). Spontaneous emergence of multiple drug resistance in Tuberculosis before and during therapy. *PLoS Med*. 6. 23 - 31

Dye, C., Garnett, G.P., Sleeman, K., & Williams, B.G. (1998). Prospects for worldwide Tuberculosis control under the WHO DOT strategy. *Lancet*. 352(9144).1886–1891.

Gelman, A., Van Mechelen, I., De Boeck, P., Leenen, I., & Poblome, j., (2000). Bridges between deterministic and probabilistic models for binary data.AvailableSSRN:http://ssrn.com/abstract=1010110orhttp://dx.doi.org/10.2139/ssrn.1010110. Accessed on Jan 2012.

Gillespie, S. H. (2002). Evolution of drug resistance in mycobacterium Tuberculosis: clinical and molecular perspective.*Antimicrobiology and Agents Chemotherapy.* 46. 267–274.

Hsie, J. Y., & Bryson, V. (1950). Genetic studies on the development of resistance to neomycin and dihydrostreptomycin in Mycobacterium .*Tubercle.* 62. 286-299.

Iwasa,Y., Michor, F., & Nowak, M. A. (2004). Evolutionary dynamics of invasion and escape. *Journal of Theorical Biology*. 226. 205-214

Iwasa,Y., Michor, F., & Nowak, M. A. (2003). Evolutionary dynamics of Escape from biomedical Intervention..*Proc Roy Soc* 270. 2573-2578

Karlin, S., & Taylor, H. M. (1975). A First Course in Stochastic Processes. Second Edition. Academic Press. London

Luria, S., & Delbruck, M. (1943). Mutations of bacteria from virus sensitivity to virus resistance. *Genetics*. 28- 491

Lea, D., & Coulson, C. (1949). The distribution of the numbers of mutants in bacterial populations. *Journal of Genetics.* 49. 264-285.

Mariam, S. H., Werngren J., Aronsson, J., Hoffner, S., & Andersson, D. I. (2011). Dynamics of antibiotic resistant *mycobacterium tuberculosis* during long-term infection and antibiotic treatment. *PLoS ONE* 6(6): e21147. doi:10.1371/journal.pone.0021147

Mel, S. (2012). Global TB Control Report. *TB Alliance News Letter***.**

Musser, J. M. (1995). Antimicrobial Agent Resistance in Mycobacteria: Molecular Genetic Insights. *Clinical Microbiol*. Rev.;8:496–514

Ribeiro, R. M., & Bonhoeffer, S. (2000). Production of resistant HIV mutants during antiretroviral therapy. *PNAS*, 97, 7681-7686.

Gagneux, S., Clara, D. L., Peter, M. S., Tran, V.**,** Gary, K. S., & Brendan, J. M. B. (2006). The competitive cost of antibiotic resistance in mycobacterium tuberculosis. *Science.* Vol. 312 no. 5782 pp. 1944-1946

Ross, S. M. (1996). Stochastic Processes. Second Edition. John Wiley & Sons. Inc.
Shimao, T. (1987). Drug resistance in Tuberculosis control.*Tubercle.* 68:5–18.
Schinazi, R. B. (2006). The probability of treatment induced drug resistance. *Acta Biotheoretica*. 8.234-241
van Helden, J. (2003). Prediction of transcriptional regulation by analysis of the non-coding genome. *Current Genomics*. 4. 217-224.
Warner, D. F., & Mizrahi, V. (2006). Tuberculosis chemotherapy: the influence of bacillary stress and damage response pathways on drug efficacy. *Clin. Microbiol*. Rev. 19, 558–570.
WHO. (2013). Global Tuberculosis report. *WHO Report.* France
WHO. (2010). Multidrug and extensively drug-resistant TB: Global report on surveillance and response.*WHO 2011 Report* **.** France
WHO. (2009). Global Tuberculosis control – Epidemiology, Strategy, Financing. *WHO Report 2009***.**Switzerland
WHO. (2007). The Global MDR-TB & XDR-TB response plan.*WHO Report*.Switzerland
Zheng, Q. (1999). Progress of a Half century in the study of the Luria Delbruk Distribution. *Mathematical biosciences*. 162. 1-32

Appendix1: R codes

```
x=0:100
> p=dbinom(0:100,39062500, 2.56*10^-8)
> q=dbinom(0:100, 4444444444, 2.25*10^-10)
> par(mfrow=c( 2,2))
>plot(0:100,dbinom(0:100,39062500,2.56*10^8),,type='l',col="red",xlab="Containment load of 39062500",ylab="probability",main="Prob Dist of INH Resistance",sub="Fig 1. No. of Drug Sensitive MTB strain")
>plot(0:100,dbinom(0:100,4444444444,2.25*10^10),,type='l',col="red",xlab="Containment load of 4444444444",ylab="probability",main="Prob Dist of RIF Resistance",sub="Fig 2. No. of Drug Sensitive MTB strain")
>plot(0:100,dbinom(0:100,10^10,2.56*10^8),,type='l',col="blue",xlab="clinical bacterial load of 10^10",ylab="probability",main="Prob Dist of INH Resistance",sub="Fig 3. No. of Drug Sensitive MTB Strain")
>plot(0:100,dbinom(0:100,10^10,2.25*10^10),,type='l',col="blue",xlab="clinical bacterial load of 10^10",ylab="probability" ,main="Prob Dist of RIF Resistance", sub="Fig 4. No. of Drug Sensitive MTB Strain")
```