Mycobacterium tuberculosis: Telling a Story of Truths

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Mycobacterium tuberculosis **is a lung disease which is extremely fatal if one acquires it in the air and fails to acknowledge and treat it immediately. There are currently numerous studies that are being carried out to develop new drugs to treat the disease as well as new technologies to improve present testing methods. This essay is unique in that it tell a very informative story about tuberculosis and looks at what still needs to be achieved in order to treat patients and thus prevent public and/or hospital-aquired transmissions.**

Key words: *Mycobacterium tuberculosis,* Drug susceptiblity tests, Incidence and Prevalence.

Mycobacterium tuberculosis (TB) – a disease of the lungs. It is a pandemic as dreadful as HIV/AIDS, but spread through respiratory droplets in the air. Its spread is prevented by forming a tubercle in the lungs (Alexander and Strete, 2001) .

There have been instances, however, where people have became severly affected by TB due to poor health and immunosuppression. This happens because poor health favours the bacterium in the tubercle to escape and to spread to new, unaffected tissues (Alexander and Strete, 2001) .

As part of the living species, we mammals experience quite different difficulties as opposed to our plant counterparts. Rurals, for example, form a major part of the TB-infected population because of the costs involved in treating this dreadful disease. As a result, the psychological toll that the poor experiences, is often enough to forfeit all hopes of living. And with the lack of treatment the bacterium eventually becomes antibiotic resistant making treatment even more difficult. Parallel to this, family obligations and companies that adopt TB worker policies, cause infected patients to present themselves for tertiary treatment (R. Singh, University of KwaZulu-Natal, South Africa, personal writing).

The resistance of TB to a wide range of antibiotics is a result of the high frequency of mutations which the bacterium acquires when treatment fails or is given late. This causes multi-drug resistant (MDR) and extensively drug-resistant (XDR) tuberculosis on a global spread, hence the emphasis on its enormity in the first-line drug-resistance global project survey established by the World Health Organisation (WHO) and the International Union Against Tuberculosis and Lung Disease (IUATLD) in 1994 (WHO, 1997, 2000, 2001). The report shows China having the second highest annual number of new TB cases, with Kazakhstan and South Africa being at the forefront having an incidence 3 times the TB cases found in China. Personally, I think the huge population numbers in those countries are the reason for the high incidences and I am sure that the increase in the prevalence trend for Kazakhstan confirms the incidence data.

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On a continuous basis, scientists are trying to find new methods of diagnosing TB by researching and testing old drugs in hope of developing, better and, new versions of drugs.

Presently, TB is controlled by early screening and surveillance followed by first-line and second-line drug therapy (Zhang *et al*, 2002; Chopra *et al*, 2003). However, mutant TB strains are very difficult to treat with selective drugs (antibiotics) since they are resistant (Starr and Taggart, 2001). The resistance of TB to second-line drugs such as para-amino-salicylate, kanamycin, cycloserine, ethionamide, amikacin, capreomycin, thiacetazone, fluoroquinolones) are poorly documented in the literature because there is no standard set of tuberculosis isolates that have been susceptible to these drugs. The contrasting view is true for first-line drugs like isoniazid, rifampicin, prazinamide, ethambutol and streptomycin (Espinal *et al,* 2001; WHO, 2001; Rom *et al*, 2004)

The fact that certain bacteria become resistant to particular antibiotics re-interates Darwins theory of Natural Selection (Starr and Taggart, 2001). For example, streptomycin and cycloserine are antibiotics that bind with some bacterial proteins and inhibits their activity. For further information on drug-binding proteins please refer to Zhang and Amzel (2002). In some strains of variant bacteria, mutations alter protein conformatoins causing antibiotics to fail binding to them. In this case the mutant bacteria escapes the potent effects of the drug and invades the body to a greater extent. Such antibiotic resistant strains are making it difficult to treat TB, gonorrhoea, staphylococcal infections and many others. Therefore there is a need for antibiotics to evolve so that they overcome the defenses by a selection process. This can only be achieved if parts of the antibiotic are modified so as to make it more effective. This has already been done by drug companies for many drugs, one of them being streptomycin (Starr and Taggart, 2001).

In the human body vitamin B6 is not nutritionally required in large quanitities and therefore its deficiency is seldomly noticed. During TB treatment, this deficiency is induced by many drugs and poisons that act on the aldehyde group of the vitamin B6 thereby sequestering the enzyme. Isoniazid, an anti-tuberculosis agent, reacts covalently to make vitamin B6 unavailable for phosphoylation by pyridoxal kinase. This happens because *Mycobacterium tuberculosis* contains low levels of kinase causing its growth to be effectively blocked by the agent (Woehl and Dunn, 1995). In cases where the diet are supplemented with vitamin B6, prolonged treatment with isoniazid would not generate a B6 deficiency in the patient by the same mechanism because there would be very little or no aldehyde groups to act on.

MDR-TB is the initial cause of placing limitations on preventative and therapeutic measures, and it is also the reason for developing drug-susceptibility tests (DST) (Kim, 2005). DST are performed using several methods to cultivate TB. These are commonly employed by Supranational Reference Laboratories (SRLs) and include solid media (Middlebrook and Lowenstein-Jensen (LJ)), Broth-based growth system (Bactec 460), and the MGIT (Mycobacterium Growth Indicator Tube). For further descriptions on these testing methods read: Snider, Jr. et al., 1981; Siddiqi et al., 1985; Tarrand and Groschel, 1985; Rastogi et al., 1989; Rom et al., 2004; Sharma and Mohan, 2007.

However, the accuracy of these methods/ techniques are very imperative in order to obtain good susceptibility patterns, and almost impossible to achieve in third world settings. Macrodilution methods have been the focus over the last five decades or so, with smaller DST systems becoming neglected. It is experiementally assumed that this is possibly because of the risk factors involved in using microdilution methods (R. Singh, University of KwaZulu-Natal, South Africa, personal writing, readings and observations).

DST such as the determination of minimum inhibitory concentrations (MICs), is affected by medium pH, incubation temperature, time and the presence of antagonistic substances in the medium that may affect drug performance (Kim, 2005). In the case of second-line drugs, the test environment confers instability to the drug and thus makes it difficult to administer to MDR-TB infected patients with a high degree of confidence (Kim, 2005).

The use of second-line drugs in combination therapies are common. David (2001) had investigated the activity of D-cycloserine and β-chloro-D-alanine against M. tuberculosis, and

he found that ß-chloro-D-alanine reduced the MIC of D-cycloserine (i.e. synergic effect). In conjunction with this studies have also been conducted on the *in vitro* bactericidal activities of antimicrobial agents combinations in STD/AIDS-infected patients. However, with the emergence of MDR-TB, the optimization of second-line drugs for administration becomes crucial, since combination therapies are extremely expensive. However, this is a difficult endeavour because in order for these drugs to be optimized, their toxic effects have to be reduced while maintaining effective dosages (R. Singh, University of KwaZulu-Natal, South Africa, personal deductions).

'The disturbance of strain patterns during subculturing relative to utilizing primary cultures, heat-induced conformational changes of drug-binding proteins, poor accuracy and precision on calculating drug potency' (Kim, 2005; Sharma and Mohan, 2007) the numerous problems associated with the standarisation of tests and the stability of the drugs in different culture media makes DST time consuming and costly (Martin-Casabona *et al.,* 1997; Victor *et al.,* 1997).

There has not been a single report published in tuberculosis research about that fact that the active compounds of drugs are possibly degraded due to the process of oxidation. Through personal observation, I found the probability of this occurring quite high, especially when drugs are being weighed for performing MIC tests. Moreover, partial hydrolysis induced by repeated freeze-storage and retrieving cycles, may affect drug potency too (British Pharmacopoeia, 1993). This may reflect ambiguity in the results if the experiment is replicated.

Since oxidation affects the activity of drugs, I suggest that, in the future, the potency of anti-tuberculosis agents be established by performing a back-titration (British Pharmacopoeia, 1993), prior to calculating it (rather than determining the toxicity based on the instruction label. However I would suggest to other researchers that they should first check when the drug was purchased and for how many years the drug is active for) because routinely purchasing these drugs is not cost-effective. This provides evidence that the efficacy of drugs cannot really be compared in a meaning way. This makes the calibration of DST methods a huge effort.

Furthermore, there should be more studies conducted on isolates that are collected from both tuberculosis cases who fail treatment with second-line drugs and patients who have never been treated with those drugs (Petrini and Hoffner, 1999; Sharma and Mohan, 2007). This is the reason as to why there are often reports on the strong correlation between first-line DST and clinical responses and very seldomly any results for second-line DST as in the case of cycloserine, for example (R. Singh, University of KwaZulu-Natal, South Africa, personal deductions).

Also, scientists should look into cutting the time spent performing tests by comparing testing methods and techniques with results obtained from previous studies. For example, Sethi *et al* (2007) tested 50 isolates of M. tuberculosis in the proportion and BMM methods to first-line drugs. They found that the Broth Microdilution Method (BMM) showed results that could be read earlier (14 days) as compared to the proportion method (21 – 28 days). Since *M. tuberculosis* grew earlier in broth than solid medium it was a rapid method which saved 2 weeks. Therefore it is common sense for scientists to opt for BMM rather than for solid medium. It is also a great advantage since there were no significant differences in the susceptibility results between the 2 methods.

The questions that arise from Sethi *et al*., 2007 and which has never been asked are: 1. Could there be any changes with second-line drugs? 2. If so, what are the differences and statistical implications? 3. Why do these differences occur? and, 4. What gives rise to these differences?. These questions could be attempted in the future when concurrent experiments involving MIC for first-line and second-line drugs are determined at the same time.

Once the technical difficulties associated with DST are dealt with individually (see small print), it would eventually result in the development of 'gold-standard' MDR-TB isolates for second-line drugs as there are for first-line drugs. This would serve as the basis for the standardization of the testing methods that are used for drugs exclusive to treating XDR-TB. This is because one cannot isolate XDR-TB without taking MDR-TB into account.

This would allow for individual case management and also for drug-resistance

surveillance, that could be included on the WHO/ IUATLD. It is necessary to know the drug susceptibilities of individual patient's strains to make sure an appropriate combination drug is given.

The work required to be done in order to cure tuberculosis extends far beyond our reach. It is like fighting a losing battle. Hope is all we have, however, it is disappointing that developing countries would suffer and that the democracy that Africa has built for itself be blown away like dust. I hope that my article has an impact on the TB community and that one realises that fighting TB is more than just a microbiological aspect. I hope this essay helps get closer to 'eliminate' this nasty disease, hopefully for good.

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