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EXTENSIVELY DRUG-RESISTANT TUBERCULOSIS: THE ROLE OF PRIMARY TRANSMISSION IN KWAZULU NATAL, SOUTH AFRICA

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EXTENSIVELY DRUG-RESISTANT TUBERCULOSIS: THE ROLE OF PRIMARY TRANSMISSION IN KWAZULU NATAL, SOUTH AFRICA

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Bachelor of Science

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Abstract

EXTENSIVELY DRUG-RESISTANT TUBERCULOSIS: THE ROLE OF PRIMARY TRANSMISSION IN KWAZULU NATAL, SOUTH AFRICA

By Amanda Feldpausch

Background

Extensively drug-resistant tuberculosis (XDR TB) remains a major health concern in KwaZulu Natal, South Africa. With high rates of HIV co-infection and poor clinical outcomes, prevention of XDR TB cases is imperative. Current prevention programs assume drug resistance is most often acquired as a result of incomplete or improper therapy; however, little is known of the impact of primary transmission on the epidemic. In order to inform effective intervention programs, we examined genotypic and epidemiologic data to determine the mechanism of development of XDR TB in the province.

Methods

We investigated culture-confirmed XDR TB cases diagnosed in KwaZulu-Natal province between August 2011 and April 2012. Data were collected from each enrolled case through a patient interview, medical record review, home visit, and genotyping of the XDR TB isolate. XDR TB cases were considered to be due to acquired resistance if there was evidence of previous treatment for MDR TB. Data from genotyping and epidemiologic investigation were used to transmission links between enrolled patients.

Results

A total of 140 XDR TB patients were screened for inclusion in the study from August 2011 to April 2012, of which. 107 patients were enrolled. XDR TB isolates were available were transported to our lab for 73 of these patients and 66 patients had genotyping data available to be included in the analysis. The median age was 35 years (IQR 28-45) and 36 (55%) were female; 50 (76%) patients were HIV-infected. Only 23 (35%) had history of prior MDR TB treatment. Additionally, XDR TB isolates from 58 (88%) of the 66 patients studied were genetically similar and determined to be clustered. Epidemiologic links were found between 20 (34%) of patients and other patients within their own cluster through residential information and places of social congregation. After review of all data, 62 (94%) of patients were considered to have resistance as a result of primary transmission.

Conclusions

Primary transmission appears to be the mechanism by which the majority of individuals develop XDR TB in KwaZulu-Natal. Further characterization of the transmission networks in the province may help define the focus of effective intervention programs.

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CHAPTER I: BACKGROUND

Epidemiology of tuberculosis

In 2011, an estimated 8.7 million incident cases of Tuberculosis (TB) occurred worldwide. TB is often thought to be an archaic disease because of the perceived low-impact in developed nations, however, it remains among the leading causes of death in women and HIV infected individuals in low- and middle- income countries worldwide with more than 95% of TB deaths occurring in these nations. TB can be a debilitating disease, and with an estimated 1.4 million deaths per year, it can have a major effect on the health and economy of areas where TB incidence is high(1, 2). Africa is second only to Asia in the share of TB disease with 26% of all infections occurring in the region. South Africa has been included in the 22 High Burden Countries (HBC) targeted by the WHO; these countries, together, account for an estimated 82% of incident cases worldwide. Additionally, South Africa, is listed among the five countries with the largest number of incident cases with an estimated 500,000 incident cases of tuberculosis in 2011, 330,000 of which are in HIV infected individuals. This translates to a rate of 993 cases per 100,000 population with 65% of incident cases being among HIV infected individuals(2).

Epidemiology of Multidrug-Resistant and Extensively Drug-Resistant TB

There were an estimated 500,000 incident cases of multi drug-resistant tuberculosis (MDR TB) globally in 2011 accounting for approximately 3.7% of all new TB cases and 20% of previously-treated TB cases(2, 3). In 2008, WHO received reports from 33 countries citing confirmed extensively drug-resistant tuberculosis (XDR TB)

cases. Together, these countries reported 963 incident infections representing 5.4% of the MDR TB cases that year(1). In 2011, the proportion of XDR TB cases among MDR TB diagnoses was estimated to be much higher at 9.0%(2). Since 1994, 134 countries have performed surveillance and provided data on drug-resistant tuberculosis(1, 2). It is estimated that 60% of the MDR TB cases worldwide are in India, China, the Russian Federation, and South Africa(2).

MDR TB is defined by resistance to Isoniazid and Rifampicin, two of the most potent first line anti-TB therapy drugs. Those with MDR TB may also be resistant to other first line drugs, but it is not necessary for diagnosis. Those who are resistant to Isoniazid and Rifampicin, any flouroquinolone, and to a second line injectable are considered to have extensively drug-resistant tuberculosis (XDR TB)(4). In South Africa, 80% of TB cases with a drug-susceptibility test result were found to have at least MDR TB(2).

Drug resistance adds complication to an already complex and time intensive treatment schedule. Drug susceptible TB requires 6-9 months of treatment with first line medications. Drug resistance requires the use of second-line medications, which are less potent and require a longer duration of treatment. Drug-resistant TB treatment can be more than 2 years with the ultimate temporality dictated by several biologic and behavioral factors. Degree of resistance of the patient's strain of TB, patient adherence to drug regimen, and response of the patient's particular strain of drug-resistant TB to the anti-TB therapy all play a role in dictating length of treatment. In addition to treatment duration and number of drugs, second line treatment includes an injectable drug. This

adds a health systems and logistics complication; a health professional must be present to administer the injection and the patient must be able to make the trip to the facility(4).

Tuberculosis and HIV co-infection

TB is the leading cause of mortality in those infected with HIV, accounting for over 25% of deaths(1, 2). Worldwide, an estimated 34 million people were living with HIV in 2010 with 67% of them in sub-Saharan Africa(5). TB/HIV co-infection is a major cause of morbidity and mortality in South Africa, with a disproportionate effect in the country due to the co-epidemics of the two diseases(6).

Of the 8.7 million estimated TB infections worldwide in 2011, 13% or 1.13 million were estimated to be co-infected with HIV. Within the African Region, a startling 39% of cases are estimated to be co-infected with HIV, and within South Africa that estimate rises to 65% or more(2). This can be partially attributed to the HIV epidemic in South Africa, the largest in the world with more than 5.6 million people estimated to be living with HIV in the country(5). When comparing 9 high burden African countries, an association of an increase of 13 TB cases per 100,000 population with every 1% increase in HIV prevalence was identified indicating a synergy between the two epidemics(7).

Persons with HIV are at increased susceptibility of developing active TB disease more quickly than those who are HIV negative, with some HIV-positive patients progressing to active disease in as little as 4 weeks from exposure(8, 9). This may be attributed to reduced immune function in those patients with HIV, allowing *M. tuberculosis* bacilli to proliferate without the mediation of a healthy immune response. Additionally, HIV-positive persons are 20 times more likely to develop active TB disease

from latent tuberculosis infection (LTBI) than those who are not HIV-positive. HIV-negative persons with LTBI have a 5-10% lifetime risk of transitioning to active TB if they are not treated; in contrast, HIV-positive persons with LTBI have a 5-10% yearly risk of transitioning from LTBI to active TB(10). This has been demonstrated in prospective studies on HIV-positive individuals where a statistically significant association was found between being HIV infected and progressing to active disease from latent infection, with CD4 count and being anergic increasing that risk(7-10).

The HIV epidemic has likely also contributed to the increase in prevalence of MDR TB. A systematic review of studies and surveillance data show that outbreaks of MDR TB have primarily occurred in HIV-infected individuals(11). Additional links are shown by studies done in the US and other industrialized countries among persons with MDR and XDR TB. One such study in the US concluded that HIV infection was 3.76 times higher in XDR TB cases than in drug-susceptible TB cases (95% CI 2.25-6.30)(12). In the late 1980's and early 1990's, several outbreaks of MDR TB were identified in industrialized countries and all were associated with HIV-infection. In these cases, mortality was >70% and occurred quickly (4-8 weeks) after onset of TB disease symptoms(11). High rates of drug-resistant tuberculosis in a community with a high HIV prevalence may indicate concern for increased morbidity and mortality in the area.

Although MDR TB does not cause disease more often than drug susceptible TB in HIV-infected persons, HIV infection has been shown to be associated with malabsorbtion of anti-TB drugs which has implications for acquired resistance. Low CD4 count is associated with acquired resistance; in a study done on HIV-positive patients with drug-

susceptible TB disease, researchers saw acquired monoresistance to rifamycin occur more often in patients with a median CD4 of 16 versus the median CD4 count of 144. Levels of pyrazinamide and rifampin were measured in individuals within the study, absorption of the two drugs was found to be significantly lower in patients with a low CD4 count when compared with absorption in those with a high CD4 count ($p = .03$ and $p = .01$ respectively). Low absorption indicates that the person may not be receiving an adequate amount of the drug to effectively kill the bacterium, which may select for additional resistance as evidenced by the observed acquired resistance to rif. (13, 14).

In addition to implications for increased risk of acquired resistance, HIV-infection and low CD4 count is also associated with poor outcomes in TB patients. In a study focusing on this relationship, 27% of patients with low CD4 count (<200 cells/μL) experienced death or failure as opposed to only 11% of HIV-positive patients with higher CD4(13, 14). Outcomes for TB also vary by access to quality care and treatment, with effective management of a specific patient being an integral part of achieving completion of treatment and cure.

Drug-resistant Tuberculosis management

In a middle-income country like South Africa, management of drug-resistant TB is a difficult task. In South Africa, in 2007, the average time between sputum collection and start of treatment was 16 weeks. The delay is attributed to the time it took for sputum results to be produced (4-6 weeks) followed by significant lag in receipt of the results by the facility due to poor communication means. Facilities need to then locate the patient who may have moved or given an inaccurate address when the sample was taken(15).

During the interim between DST testing and results, those infected with resistant strains are often receiving inadequate treatment, or they may be receiving no treatment at all. TB patients receiving inadequate treatment may remain infectious until effective treatment is prescribed. Delays in DST results, therefore, may have implication for spread of DR TB through primary transmission.

In addition to delays in DST results, WHO reports that there were over 10,000 notified cases of MDR TB, but only 5,600 enrolled in MDR TB treatment(2). Though some of these patients may be deceased at the time of DST result, many are alive and not treated for other reasons (not located, refuse treatment, default, etc.) and remain infectious in the community. The populations that are most effected are also a reason for concern; studies show that homelessness, imprisonment, alcohol consumption, and unemployment are associated with acquisition of MDR TB. Additionally, persons of low socio-economic status (SES) are more likely to experience death, failure, or default of treatment(12, 16). As previously discussed, MDR and XDR TB are associated with high mortality, especially in populations where HIV is highly prevalent. In a studies done to assess the cause of death in HIV infected individuals, autopsies showed TB present in up to 79% of the deceased(17, 18). WHO estimates that 13,000 incident cases of MDR TB occur each year in South Africa, however, additional studies also suggest that MDR TB is more widespread in rural areas than previously thought(1, 19-21). These associations raise concerns in a province like KwaZulu Natal where HIV prevalence is high and general SES is low.

Outcomes for tuberculosis treatment

Outcomes for treatment of MDR and XDR TB are different from that of susceptible TB. Studies show that the risk of poor outcomes in MDR and XDR TB patients is significantly higher than that of drug-susceptible TB patients. In an example of 83 cases of XDR TB in the US between 1993 and 2007, 53% of which were HIV positive, 35% (26 cases) died during treatment of which 81% (n=21) were HIV positive. XDR TB cases were found to be 1.82 times more likely to die during treatment than MDR TB cases (95% CI 1.10 – 3.02), and 6.1 times more likely to die during treatment than drug susceptible cases (95% CI 3.65-10.20)(12). Additional studies show similar results, with XDR TB patients exhibiting a two-fold risk of death above MDR TB patients and a six-fold risk above drug-susceptible TB patients(19, 22). In HIV negative individuals, treatment success in XDR TB patients with aggressive treatment schedules and strict drug adherence is shown to be 60%(23). In the study from the US previously mentioned, 40 XDR TB cases (66%) converted to sputum culture negative while in treatment. Median time to culture conversion was 183 days (IQR 104-344). This was significantly less likely than culture conversion in MDR TB cases (PR, 0.57; 95% CI 0.34-0.99) and drug susceptible cases (PR, 0.55; 95% CI 0.33-0.94)(12). Although these patients reached culture conversion, this does not indicate successful treatment, or cure, of XDR TB. In the same study, only 44% of XDR TB cases were documented to have completed treatment, in other studies, percentages of successful treatment are closer to 30% in XDR TB patients(12, 22). HIV status is also a factor in MDR and XDR TB treatment outcome. HIV-infected individuals are more likely to experience death as an outcome than non-infected individuals(12).

Treatment of drug-susceptible TB is an arduous task, and, as previously discussed, MDR and XDR TB treatment is even more complicated. Second-line drugs are less potent against tuberculosis than first-line which necessitates use for a longer duration of time(24). Length of treatment will vary given a patient's response to treatment and can last for over 2 years. Knowledge of complex treatment, early diagnosis, and regular testing through culture and Drug-Susceptibility Testing (DST) to monitor success of treatment regimen is necessary(4, 25).

Adverse treatment events

Second-line drugs are also linked with toxic side effects and morbidity, some of which cause permanent or irreversible disability. Although second-line drugs are less potent, the adverse events associated with them are often severe. Studies have shown that some of the most common and mild adverse events include gastrointestinal disturbances in up to 100% of cases as well as dermatological issues in 4.5 – 43.3% of patients. More serious events include ototoxicity (damage to the ear which may result in permanent loss of hearing) which has been observed in up to 42% of patients, psychiatric disorders were commonly seen in 18 – 21.3% of patients, arthralgia (joint pain), epileptic seizures, and hepatitis(12, 26, 27). These adverse events have also been shown to be more common in HIV infected individuals(28). Despite the occurrence of adverse events, no patients discontinued anti-TB therapy in totality; instead, a certain drug was replaced or suspended in cases when possible (12, 26, 27). As resistance increases, fewer drug options are available for use in the place of a drug which produces negative side-effects in a patient. This serves to illustrate the importance of proper diagnosis and treatment early in the course of the disease.

Keeping these adverse events and outcomes in mind, it is clear that the development of an effective treatment regimen for a case of XDR TB is imperative. WHO provides guidelines on this process which they outline in groups. Group 1 drugs, or first-line oral agents, which include isoniazid, rifampicin, ethambutol, pyrazinamide, and refabutin are the most potent drugs with the fewest and least severe adverse events. These therapies should be used if DST results indicate that a particular first-line drug may be effective against the patient's strain of XDR TB. Previous failure with this group of drugs should be considered in conjunction with DST results. For a patient with any resistance shown through DST, at least one of the group 2 drugs, the injectable drugs, should be used. These drugs include kanamycin, amikacin, capreomycin, and streptomycin. Resistance to streptomycin is common, so kanamycin or amikacin are recommended as the first choice of these drugs. Group 3 drugs should also be used if there is any drug-resistance. This group is made up of the flouroquinolones: moxifloxacin, levofloxacin, and ofloxacin. Group 4 drugs are the oral bacteriostatic second-line agents, ethionamide, protionamide, cycloserine, terizidone, and p-aminosalicyclic acid. These agents tend to have high association with adverse events, but are necessary in the treatment of XDR TB. Group 5 drugs are agents with unclear efficacy and are not recommended for routine use in treatment. However, it may be necessary to incorporate these therapies when other treatments fail. Standard procedure exists for developing a treatment regimen for DR TB based on these 5 groups; these regimens are two-phased, the first phase including an injectable drug and the second phase excluding it. The actual drugs used within the regimen should be individually based on the patient's specific DST results and subsequent reaction to the drug regimen prescribed(29).

Some of the drugs within each group are more effective than others, unfortunately, cost is often the determining factor for which drug will be used as opposed to the efficacy. WHO recommends that later-generation flouroquinolones rather than early generation should be used, however, later-generation drugs are often more costly and unavailable to patients in developing countries(29).

After the drug regimen has been chosen, an intensive phase of greater than or equal to 8 months is recommended with a total duration of treatment greater than or equal to 20 months. The peak for cure in patients who have never been previously treated for MDR TB is between 18.6 and 21.5 months as opposed to a peak of 27.6 to 30.5 months for those who have been previously treated for MDR TB. Culture conversion is an important factor in determining the appropriate end to treatment; patients should have three consecutive negative cultures before being considered cured(4).

In addition to anti-TB therapy regimens, ART is recommended for all patients with HIV and drug resistant TB requiring second-line drug treatment. This is a blanket recommendation regardless of CD4 cell count. Despite recommendations, WHO reports that evidence is lacking on the best drug regimens for XDR TB(4, 29).

Mechanisms for development of drug-resistance

Development of drug resistance in *M. tuberculosis* evolves primarily through spontaneous mutational events, selection of these mutations is a result of human activity and did not exist before anti-TB drugs were introduced(30). For resistance to form, a subpopulation of resistant bacteria must survive initial treatment. Spontaneous mutations will occur in the patient's strain of TB regardless of treatment regimen. If the patient is

treated with a regimen which does not contain a drug to which the mutated bacilli is susceptible, then that particular bacteria will survive and proliferate. This occurs when patients are treated with inappropriate or inadequate treatment regimens or when patients are selectively non-compliant in drug adherence(31).

Development of MDR TB based on this drug pressure is a stepwise process (30). Drug pressure most often occurs when a patient is being treated with drugs to which their strain of TB is already resistant. This lowers the effectiveness of the overall treatment and can cause resistance to the drugs in the regimen that the patient was not originally resistant to. For example, if INH mono-resistant TB is treated with INH and RIF, the insufficient treatment will select for the development of RIF resistance(32). Although single drug treatment is not recommended, risk of drug resistance with this type of therapy is extremely high(30). WHO treatment guidance prescribes for empiric treatment of MDR TB when first line drugs fail as opposed to the addition of streptomycin only which may lead to more resistance depending on the patient's initial resistance pattern(4).

Systematic deficiencies in treatment regimens can lead to resistant strains that persist in a community. Specifically in the province of KwaZulu Natal, South Africa, over the course of slightly more than a decade, a local strain of *M. tuberculosis* evolved to develop resistance to 7 anti-TB drugs. Genotyping commenced in 1994, after which the strain was compared over time. Ethambutol resistance was first observed in 1995 followed by resistance to second-line drugs observed in 1997. Resistance to ethionamide was first seen in 1997, then capreomycin in 1998, kanamycin in 1999, and flouroquinolones in 2000. This fingerprint was found in a large number of patients in Tugela Ferry in 2005 and continues to persist in the province(31).

Though drug resistance originated from drug pressure, once resistance is present within a strain that resistance will be passed on to secondary contacts who develop active TB disease. Resistance as a result of this primary transmission is a cause for concern.

Determining mechanism of cause for drug-resistant tuberculosis

An important step in determining whether a case of tuberculosis is the result of acquired resistance or transmitted resistance is genotyping of the isolate. The genotyping results will allow the researcher to compare one patient's strain with another patient and look for strains that are genetically related. Those strains which are likely to be a result of primary transmission may be closely related in their genotyping pattern. However, strains may continue to mutate once they have been passed to a new patient, therefore, not all primary transmission cases will have strains that are very closely related. There are three main types of genotyping, restriction-fragment length polymorphism (RFLP), mycobacterium interspersed repeat units (MIRU), and spacer oligonucleotide typing (spoligotyping).

The historical approach to genotyping *Mycobacterium tuberculosis* is RFLP. This method is used to analyze the distribution of insertion sequence IS6110 within the isolates. Strains that are epidemiologically linked will have identical RFLP patterns while those that are not linked will have different patterns. RFLP typing requires sub-culturing of isolates for several weeks to obtain sufficient amounts of DNA and requires supplementary genotyping methods to increase discrimination by mapping specific mutation sites(33).

The mycobacterium interspersed repeat units (MIRU) are abundant in the *M. tuberculosis* genome. The use of MIRU genotyping identifies the number and size of repeats in each of 12 units. MIRU testing is performed through PCR (polymerase-chain-reaction) assay and gel electrophoresis. The mapped repeats will vary in length and structure but, like RFLP, may be identical in isolates that are linked or transmitted instead of acquired. MIRU is typically less complicated and time consuming than the IS6110-based RFLP testing and may be automated to evaluate large numbers of strains. Additionally, it does not require the time-intensive DNA purification previously noted in RFLP testing(33).

Finally, spoligotyping, allows the researcher to look at the direct-repeat locus in *M. tuberculosis* and evaluate spacers between the 36-bp direct repeats. The spacers will have different patterns in strains that are not epidemiologically linked. Only a small amount of DNA is required for this method. Spoligotyping is less discriminatory than RFLP and therefore should be combined with other methods to increase the probability of proper identification of epidemiologic links(33).

Although all three techniques are employed in studies today, RFLP was the method used in major studies of tuberculosis outbreaks and drug-resistance in the US in the early 1990's and continues to be used (8, 34, 35). RFLP is also the technique employed in this study. Genotyping is just one step in the process of identifying transmitted cases of TB. We should consider the information from genotyping tests in conjunction with epidemiological and social network data to determine if physical links exist between patients with identical isolates(33).

Epidemiologic investigation and Social Network Analysis

Links between cases may be made through the genotyping procedures described above, however, the next step in determining if a case of TB is likely to be the result of transmission will include attempts to establish physical or epidemiological links between cases. Genotyping of *M. tuberculosis* strains has been conducted in several studies with the use of epidemiologic investigation to establish these links(36-38)..

Epidemiologic investigation involves contact tracing; examining persons which the TB patient has been in contact with and looking for links between TB patients as well as contacts who may have active TB and may have passed TB to the original patient of interest. Social Network Analysis takes that a step further; including places of social congregation, home location, travel means to form an entire network of persons and locations that may be associated with transmission. Social Network Analysis can be a powerful tool in linking cases of TB to one another in determining likelihood of transmission versus acquisition. This is especially important in cases of drug-resistant tuberculosis due to the severity of the disease and the urgency in determining effective prevention programs. In the past, social network analysis has been primarily used in the study of sexually transmitted infections with the analysis being done on sexual networks, however, the concept can be applied to TB networks as well(39). Though not common, social network analysis has been used in the investigation of tuberculosis networks and genome sequencing in the past. One example includes an outbreak of TB in British Columbia, Canada in 2006. Researchers were able to link cases through Social Network Questionnaires which identified common social settings between infected individuals(40). Social network analysis has an advantage over traditional epidemiologic

investigation as it considers not only persons that the diseased individual may have come into contact with, but also places of social congregation within a network.

Networks are generally conceptualized visually in the use of graphs and diagraphs, this allows researchers to determine centralized persons or locations associated with cases of genetically linked TB. Performing a social network analysis with tuberculosis patients can be complicated as the disease does not require direct contact to be spread and may have long periods of latent infection before active disease is apparent. Due to the potential for extended time between exposure and development of active disease, often TB social network analysis will require patients to recall persons and places they have been in contact with for 5 years in the past. However, once that information is obtained, the researcher can use identified clusters of patients with identical strains of TB to look for common physical links between the persons and the places they have used for social congregation(39, 41). Social network analysis is ideal in the case of TB transmission as there may be instances where personal relationships between two diseased individuals are not identifiable but a common place of congregation may be discovered(39).

If social network analysis and genotyping results indicate that transmission of the TB is occurring at high rates, changes in approaches to treatment and prevention would be prudent.

Primary transmission

Using a combination of genotyping and epidemiologic investigation has often been used in the past to determine the mechanism for occurrence of drug-resistant TB in

a community. Although most intervention programs are designed with the assumption that acquired resistance is the primary mechanism behind drug-resistant TB occurrence, many studies have shown that primary transmission is likely to be an important mechanism as well. In Puerto Rico, a study was done on 14 patients in two health-care facilities; all 14 had TB with identical DNA fingerprints of which half were epidemiologically linked, indicating transmission among those patients. In the previously mentioned outbreak among US-bound immigrants from Thailand, 17 of the 24 MDR TB cases were interviewed, 13 of which were found to be linked through epidemiologic investigation. Isolates were genotyped in this case as well, out of 23 total isolates, 20 were found to be genetically similar(42). A study done in Tugela Ferry, KwaZulu Natal, South Africa investigated the incidence of transmission of MDR and XDR TB in household settings. It was concluded that incidence of transmission among household members of those with MDR or XDR TB was high; indicating that transmission of the disease is an important pathway for spread of tuberculosis. This fact has particularly alarming implications when considering the high prevalence of HIV in the region(43).

A study done in rural South Africa investigated transmission in patients with MDR or XDR TB who had been treated for drug-susceptible TB prior to presentation of resistant tuberculosis. In this case, acquired resistance would likely be assumed given the patients previous TB treatment history. Samples for 17 participants were spoligotyped from both their original, drug-susceptible isolates and their MDR or XDR isolate. Results showed that all had experienced reinfection by a different strain than their initial isolates, further supporting the hypothesis that primary transmission an important pathway in the current TB epidemic(36).

Prevention of drug-resistant tuberculosis

Prevention of drug-resistant tuberculosis will require attention to prevention of both acquired resistance and transmission, though the approach to each is different. For acquired resistance, the most effective prevention is ensuring drug adherence. Directly observed therapy (DOTS) is a method used to confirm that patients are taking and completing therapy. It involves a healthcare provider distributing and observing the patient taking their medication at every dose. Though it is labor intensive, it has been shown to reduce acquisition of resistance as well as transmission of TB disease(44).

Another approach to preventing acquired resistance is the improvement of rapid point-of-care diagnostics and drug susceptibility testing on TB isolates for those patients who have risk factors for drug-resistant tuberculosis. The South African Department of Health does not currently recommend culture and DST on all incident cases of TB, specifically excluding those who do not have a history of prior TB treatment. Patients who have some level of resistance as the result of primary transmission are sometimes treated with inadequate regimens as a result of this policy. By diagnosing TB and identifying low-level resistance more quickly and efficiently, patients may be given an effective drug regimen from the beginning of treatment. This reduces the potential for further acquired resistance due to drug pressure as well as reducing the time the patient is infectious and improves expected outcomes for treatment (45).

Testing for HIV in all patients with TB is also an important step in reducing the incidence of acquired drug-resistant TB. As discussed previously, HIV infection is associated with malabsorption of ant-TB drugs, rapid progression to active TB disease,

and poor outcomes for treatment. Knowledge of HIV status can assist medical personnel in developing the most effective treatment plan for managing both the patient's TB and HIV infections and reducing the chance for acquired resistance due to malabsorption of therapy.

Reducing transmission related drug-resistant TB cases has many of the same factors. Better rapid point-of-care diagnostics would also be important. If MDR TB or XDR TB cases are identified early, they may receive appropriate treatment which will reduce their period of infectiousness and likelihood of transmission of the disease to others. Additionally, patients may be isolated to reduce the chance of transmission of drug-resistant strains of TB to other community members. Programmatic decisions related to where drug-resistant TB patients should be treated are important in the reduction of nosocomial transmission of drug-resistant strains within hospital settings. Hospitals handling drug-resistant TB cases should have staff that is trained and familiar with treatment regimens, isolation protocols, and management of complications inherent in drug-resistant TB treatment. Technical and monetary support will be needed for these facilities as well including strong infection control programs. Finally, effective outpatient follow-up is needed for the more than 18 months of treatment required after the intensive phase. As previously discussed, patients need to receive injectable treatments and will need to have a health professional administer the injection. Adverse events and longevity of treatment can contribute to discontinuation of treatment by patients, increasing the chance for reactivation of their active TB disease(46). Community-based treatment programs have been proven to be cost-effective through reduction of transmission and reduction of hospital costs(46, 47).

Implications

Incidence of drug-resistant tuberculosis continues to hold at high rates within middle- and low-income countries. Management of TB is difficult in these areas due to lack of resources and training of healthcare personnel in the treatment of TB. Although treatment of drug-susceptible TB is often successful, poor outcomes are expected for those with MDR TB, and especially for those with XDR TB. Increased mortality is expected for those with co-infection with HIV. The most effective way to reduce mortality from XDR TB will be prevention of drug-resistant TB from the outset. Effective prevention will depend on the mechanism for development of drug-resistant TB cases within a given community.

The purpose of this study is to analyze an initial group of 66 XDR TB patients identified in the province of KwaZulu Natal, South Africa to determine if genotypic and epidemiologic links can be made between diseased persons indicating that primary transmission of XDR TB may be an important mechanism in the area. Given this transmission is occurring, we will be able to use the data to identify risk factors associated with transmission within the province through social network analysis. This analysis will include identification of persons or locations of social congregation associated with transmission of the disease, information that can be used to inform intervention strategies. If primary transmission is identified as an important pathway to XDR TB incidence in the province, this will have major implications in the focus of treatment and prevention programs and will help to inform future research.

CHAPTER II: MANUSCRIPT

EXTENSIVELY DRUG-RESISTANT TUBERCULOSIS: THE ROLE OF PRIMARY TRANSMISSION IN KWAZULU NATAL, SOUTH AFRICA

By Amanda Feldpausch

Background

Extensively drug-resistant tuberculosis (XDR TB) remains a major health concern in KwaZulu Natal, South Africa. With high rates of HIV co-infection and poor clinical outcomes, prevention of XDR TB cases is imperative. Current prevention programs assume drug resistance is most often acquired as a result of incomplete or improper therapy; however, little is known of the impact of primary transmission on the epidemic. In order to inform effective intervention programs, we examined genotypic and epidemiologic data to determine the mechanism of development of XDR TB in the province.

Methods

We investigated culture-confirmed XDR TB cases diagnosed in KwaZulu-Natal province between August 2011 and April 2012. Data were collected from each enrolled case through a patient interview, medical record review, home visit, and genotyping of the XDR TB isolate. XDR TB cases were considered to be due to acquired resistance if there was evidence of previous treatment for MDR TB. Data from genotyping and epidemiologic investigation were used to transmission links between enrolled patients.

Results

A total of 140 XDR TB patients were screened for inclusion in the study from August 2011 to April 2012, of which. 107 patients were enrolled. XDR TB isolates were available were transported to our lab for 73 of these patients and 66 patients had genotyping data available to be included in the analysis. The median age was 35 years (IQR 28-45) and 36 (55%) were female; 50 (76%) patients were HIV-infected. Only 23 (35%) had history of prior MDR TB treatment. Additionally, XDR TB isolates from 58 (88%) of the 66 patients studied were genetically similar and determined to be clustered. Epidemiologic links were found between 20 (34%) of patients and other patients within their own cluster through residential information and places of social congregation. After review of all data, 62 (94%) of patients were considered to have resistance as a result of primary transmission.

Conclusions

Primary transmission appears to be the mechanism by which the majority of individuals develop XDR TB in KwaZulu-Natal. Further characterization of the transmission networks in the province may help define the focus of effective intervention programs.

Introduction

Of the 8.7 million incident cases of tuberculosis (TB) which occurred worldwide in 2011, an estimated 500,000 were cases of multi-drug resistant tuberculosis (MDR TB) or extensively-drug resistant tuberculosis (XDR TB). In South Africa, there were 500,000 incident cases of TB with 300,000 co-infected with HIV and over 10,000 notified as MDR TB(3, 48, 49). MDR TB is defined as resistance to at least isoniazid and rifampicin, the two most important first-line anti-TB therapy drugs; patients with MDR TB may have resistance to other anti-TB therapy. XDR TB is defined as resistance to isoniazid and rifampicin, any fluoroquinolone, and to a second-line injectable drug(4).

Drug -resistance calls for the addition of treatment and health systems complications not required in the treatment of drug-susceptible TB. The need for additional drugs, injectable therapy, prolonged treatment time and drug-susceptibility testing (DST) to monitor patient response to treatment regimens is expensive and difficult to manage in a developing nation(4, 49). The management of XDR TB is even more difficult; extensive resistance to available anti-TB therapies leave few effective drugs for use in treatment regimens. Duration of treatment for XDR TB may exceed 2 years, while patients face lower probability of treatment success with high rates of mortality in those co-infected with HIV(4, 12, 19, 22).

The development of drug-resistant tuberculosis occurs by one of two pathways: acquired resistance or primary transmission. Acquired resistance forms when spontaneous mutations in the bacillary population which confer resistance are selected for by inadequate drug therapy. This may occur as a result of poor adherence, treatment with an

inappropriate regimen by the clinician, or through malabsorption of the drugs(30, 31). Primary transmission occurs once resistance proliferates in the patient. This patient may transmit TB to another person; should that person develop active TB disease, he or she will likely have drug-resistance, even if not previously treated for TB. The current focus of intervention programs is on acquired resistance, though the proportion of DR TB cases to be attributed to each of these pathways is unknown. Quantifying this information is important in developing effective prevention programs for DR TB.

Currently, the South African Department of Health does not recommend performing culture and DST on all incident cases of TB, particularly those patients with no prior TB treatment history (50). Patients with transmitted resistance are unlikely to have DST at the outset and thus, will likely receive inadequate treatment regimens, creating conditions for the development of further drug resistance. Prompt identification of DR TB cases is important to prevent further transmission to other individuals. Those with XDR TB who are receiving inadequate treatment will typically remain infectious, increasing risk of transmission to others in the community. It is, therefore, important to identify if primary transmission is occurring within the community so that prevention programs may be modified to increase effectiveness.

To determine the proportion of cases to be attributed to each of these mechanisms in a community, two methods are often employed: genotyping of isolates and epidemiologic investigation. The three leading methods of genotyping are restriction-fragment length polymorphism (RFLP), mycobacterium interspersed repeat units (MIRU), and spacer

oligonucleotide typing (spoligotyping)(33). Social networking analysis is a more robust version of epidemiologic investigation and requires the researcher to trace not only contacts of patients, as would be done in an epidemiologic investigation, but to build a network of persons and places frequented by the individual in the hopes of identifying epidemiologic links between patients whose isolates are linked through genotyping(39).

In this study, we will use these methods to determine the mechanism of development of XDR TB in patients living in the province of KwaZulu-Natal (KZN), South Africa. This knowledge will help to inform intervention programs within the province and reduce the spread of drug-resistant TB among its population.

Methods

Study design and population

This is a prospective, cross-sectional study to determine the proportion of XDR TB cases which arise due to transmission in KwaZulu Natal (KZN) Province, South Africa. We conducted patient interviews, as well as medical record review, to determine whether XDR TB cases had previously been treated with second-line TB medications. Additionally, we used genotyping and social networking analysis to identify potential links between study participants. XDR TB subjects were identified by culture and DST results from the provincial TB reference laboratory. Individuals were eligible for the study if they had culture confirmed XDR TB disease and resided in KwaZulu Natal province. Men and women as well as children were eligible. Patients were excluded if the primary spoken language was not English or Zulu, they had prior XDR TB results more

than three months before the current episode, if they began XDR TB treatment before May 1, 2011, or if they were under 18 without a parent or guardian who could converse in English or Zulu. The target enrollment was 400 XDR TB patients; here, we present a preliminary analysis for the first 77 patients with genotyping data available.

Setting

Recruitment was performed in KwaZulu Natal, a province located in the southeastern portion of South Africa sharing borders with Swaziland, Mozambique, and the Indian Ocean. HIV prevalence is high in province with estimates at 41-46% infection among those attending antenatal clinics (51, 52). MDR TB accounts for close to 2.3% of incident TB cases or close to 30 cases per 100,000 population. XDR TB accounts for approximately 10% of MDR TB cases within the province with approximately 300 cases of XDR TB diagnosed each year(15). All suspected DR TB patients in the area have isolates submitted to the provincial TB reference laboratory which are tested for resistance to isoniazid, rifampin, streptomycin, oflaxacin, and kanamycin. A single TB reference laboratory performs all cultures and DST for patients in the province.

Patient Enrollment and Interview

XDR TB cases identified by the provincial TB reference laboratory were referred to the study staff for potential enrollment. Attempts were then made to locate the patient by calling the health facility which submitted the specimen. Once a patient was located, the field team arranged to meet the patient if he or she was still living; if the patient was deceased, a meeting with immediate family members was arranged. Upon meeting the

patient (or family member), the study was introduced and informed consent was sought. Patients who were not located within three attempts were excluded from the study.

Once consent was obtained, the subjects (or family member) were interviewed to collect data on demographics and past medical history. This included histories of TB treatment, HIV status, alcohol and substance abuse. Participants were asked to provide information on close contacts, including: name, nicknames, age, sex, and whether or not the contact had TB or DR TB. Contacts were elicited from the participants' households and workplaces. Additionally, participants were asked to list frequented places of social congregation, such as schools, hospitals, churches, etc. (see Appendix II).

Blood was drawn from participants to test for HIV, CD4 count, and viral load. Geographic Positioning System (GPS) coordinates were taken at the participants' homes. Information on the composition of the home was also recorded.

Medical Record Review

Participants were asked to report any history of hospitalization in the 5 years prior to enrollment. Medical records were abstracted to obtain data related to past episodes of TB and the illness that prompted specimen collection which yielded XDR TB. Information was gathered regarding admissions and discharges for all recorded hospital stays, chest x-ray results, HIV testing, CD4, viral loads, prior TB history, the current XDR TB episode, co-morbidities, TB culture and DST results, and other medication history.

Information gathered from medical records and patients informed classification of patients as "primary transmission" or "acquired resistance." Our *a priori* definition of transmission is a patient who has no history of treatment of MDR TB, regardless of treatment outcome. XDR TB patients with any prior MDR TB treatment were conservatively considered to have acquired resistance, even though the possibility of exogenous re-infection is well established(36, 53).

Genotyping of isolates

The original XDR TB isolates from the provincial TB reference laboratory were identified and stored. Isolates were shipped to the Public Health Research Institute (PHRI) laboratories in Newark, New Jersey, USA for genotyping. Isolates underwent IS*6110* Restricted Fragment Length Polymorphism (RFLP) testing as well as DNA sequencing of resistance-conferring regions. RFLP testing was performed under standard protocol for 5' and 3' fragments within the insertion sequence IS*6110*. In order to increase the discrimination of RFLP testing, DNA sequencing was also performed. Each isolate underwent sequencing for 10 resistance conferring regions (katG, rpoB, inhA, gyrA, pncA, rpsL, rrs1, rrs2, gidB, and embB). Results of these tests were reviewed manually. Isolates were assigned to a "cluster" if there was no more than one difference found in their resistance-conferring sites or RFLP pattern. Those isolates that did not meet the definition of "clustered" were identified as "non-clustered" (Figure 1).

Epidemiologic investigation

Data collected from the patient interview were reviewed manually for the epidemiologic investigation. Names of contacts were examined for possible matches between participants. Reports of common contacts were examined for the entire group, if common contacts were found, it was then determined if the two enrolled patients were "clustered" with genetically related strains. This process was repeated with locations of homes, work place, school, hospital admissions, and specific places of interest frequented by the participants. Additionally, we used information on general congregate locations to determine statistically significant associations between the group of "clustered" participants and certain types of social settings when compared with "non-clustered" participants.

Statistical Analysis

The frequency of demographic and clinical characteristics were calculated as percentages, while age and CD4 count were reported as a median and inter-quartile range. Demographics, TB treatment history, HIV history, and work history were compared between patients who were "clustered" and those who were "non-clustered." Statistical associations were assessed using an Exact Wilcoxon two-sample test. HIV status, ARV treatment, and history of risk factors were compared using Fisher's Exact Test to determine significant associations with clustering of genetically identical isolate groups versus the "non-clustered" group. When identifying CD4 counts, results were pulled from blood drawn at time of enrollment when available as well as results from chart abstractions. CD4 counts were classified by the number of days from enrollment in

the study and categorized for analysis (Appendix I). The CD4 count closest to the date of study enrollment was used for each patient. Demographics, CD4 counts, HIV and ARV treatment status, and history of prison, health care work, or mine work were analyzed using SAS software.

The study protocol was approved by the ethics committees of Emory University, Albert Einstein College of Medicine, University of KwaZulu-Natal and the Medical Research Council of South Africa, and by the KwaZulu Natal Department of Health (DOH).

Results

Patient Characteristics

At the time of this analysis, 140 XDR TB patients had been identified and screened. Of these, 107 patients consented for participation. Reasons for non-consent were most often refusal, inability to locate the patient, a DST older than three months, or lack of appropriate family member, in the case of patient death. Of the 107 patients consented, isolates for 73 patients had been shipped for genotyping at the time this dataset was closed for analysis. Among these, 66 isolates grew and successfully underwent genotyping; 4 isolates had partial growth and had to be regrown, 1 did not grow, and 2 patients were excluded from the study before their isolates were genotyped.

The 66 patients analyzed came from 31 distinct locations in the province, with the two largest groups residing in the provincial capital of Pietermaritzburg (5 patients) and the large city of Durban (12 patients). The median age was 35 years (IQR 28-45) and 36

(55%) were female (Table 1). HIV status was available for all patients and 50 (76%) patients were HIV-infected. Forty-three of these 50 had information on antiretroviral treatment (ART) and 42 (98%) were already receiving ART at the time of enrollment.

Prior MDR TB history was available for 60 patients, of which 37 (62%) had never been previously treated for MDR TB, meeting our definition for XDR TB from primary transmission (Table 1). Of those with prior MDR TB history (n=23), the treatment outcome was cured or completed treatment for 5 (22%), treatment failure for 11 (61%), default for 1 (6%) and unknown outcome for 6 (33%).

Clustering

Fifty-eight (88%) patients were found to cluster, based on results of RFLP and sequencing results, comprising eight distinct clusters (Table 2). The largest cluster contained 34 (59%) of study participants (Cluster 1: HP81). There were 9 (16%) patients in cluster 2, clusters 3-5 contained 3 (5%) patients each, and clusters 6-8 each contained 2 (3%) patients. Of the 23 patients with prior MDR TB treatment history, 19 (83%) were a part of clusters, suggesting that their current episode of XDR TB may be due to primary transmission, rather than acquired resistance. Additionally, epidemiologic links were found between 7 (37%) of these 19 clustered patients.

We found no association between being clustered and age ($p = .43$), female sex ($p = .72$), receipt of previous TB treatment ($p = .70$), prior history of MDR TB treatment ($p = >.99$), HIV infection ($p = .67$), ARV treatment status ($p = >.99$), or median CD4 count ($p = .25$)

(table 3). A significant relationship was found between being non-clustered and ever working in a health care facility (p = .03). There were no associations found between clustering and alcohol consumption (p = .39) history of imprisonment in the last 12 months (p = >.99), ever working in a mine (p = >.99), having a household member work in the mines in the past 12 months (p = >.99), or having a family member incarcerated in the past 12 months (p = >.99) (Table 2).

Epidemiologic Investigation

Using epidemiologic investigation, we were able to establish links between 22 (38%) of the 58 clustered patients through towns of residence and places of social congregation. When examining residential information, 7 groups of patients were identified to live in the same city or town as other patients within their cluster, resulting in a total of 21 (36%) clustered patients with a link by location of residence (table 4). Additionally, 2 patients (from cluster 4) were found to have attended the same church from 2001 to 2011. Nineteen patients had a history of previous hospitalization. Among these, 17 were clustered, in 4 of the 7 clusters. The temporality of hospitalizations for these 17 patients did not indicate that patients were hospitalized prior to sputum collection for their current XDR TB episode with another XDR TB patient. No patients reported the same contact, school, work, or place of social congregation, other than churches.

Discussion

In this study, we sought to determine the role of transmission in the XDR TB epidemic in KwaZulu Natal Province, South Africa. Using medical record review, we found that the

majority of patients had no history of prior MDR TB treatment and thus, likely developed XDR TB as a result of primary transmission. Even among those with history of MDR TB treatment, more than 80% were found to be a genotypically clustered with other study participants. Consequently, the true proportion of cases arising from primary transmission is likely to be even higher than estimates based on history of prior MDR TB treatment. Genotypic analysis demonstrates that nearly 90% of the enrolled patients have an XDR TB strain closely related to those of other study participants. Epidemiologic investigation was limited in this preliminary analysis. Nonetheless, epidemiologic links were found for more than one-third of clustered patients.

Primary transmission was defined a priori as a patient who has no history of MDR TB treatment prior to XDR TB diagnosis. Given this definition, we would initially classify 43 (65 %) of patients in this study as primary transmission. However, results of the analysis show epidemiologic and genetic links between patients who have had prior MDR TB treatment as well as evidence of successful treatment in 5 of the patients. Of the 23 patients who were determined to have prior MDR TB treatment, 19 (83%) were clustered genotypically. Of those patients, 7 (37%) were found to have epidemiologic links to other patients within their cluster. Given the clustering of previous MDR TB patients, we will increase the proportion of primary transmission cases from 43 to 62 (94%). Clustered patients with prior MDR TB treatment may have failed treatment due to reinfection with XDR TB.

In this study, an effort was made to identify all confirmed XDR TB cases in the province as opposed to focusing on an outbreak or specific healthcare facility. Similar studies have found ranges of 40-56% of patients clustered which is lower than our findings of 88% based on genotyping information alone(54-56). This may be associated with the presence of a large HP81 cluster of patients which contained 34 (59%) of patients. The HP81 RFLP type has persisted in this region for the last decade. It was the pattern seen in the 2005 outbreak of XDR TB cases in Tugela Ferry, a town within the province, and has also been followed as resistance has developed within the strain over the past 10 years(19, 31). Due to the high proportion of HP81 patients in the population, it's likely that many patients involved in the transmission network for this cluster were not included in this study. They may not have sought medical care, staff may not have been able to locate them, or they may have experienced rapid death.

The epidemiologic investigation in this study identified significant links between patients through area of residence. Given the enrolled patient's or family member's need to recall persons and places over the course of a long period, data on contacts and places of social congregation may be less robust than residential information. Few links were found in places of social congregation. This may be due to recall bias or it may be that two people linked genetically are linked epidemiologically by an intermediary case that was not identified by the study. In a study done in San Francisco, 191 of 473 TB patients were found to be clustered through genotyping; however, the epidemiologic investigation was only able to establish links between 10% of the patients(56). Consideration of both

genotyping and epidemiologic data in this study indicate that primary transmission is happening at a high rate.

In other studies, genotyping in 4 provinces (Gauteng, Western Cape, Eastern Cape, and KwaZulu Natal) show that increased levels of resistance are associated with less strain diversity, indicating that primary transmission of MDR TB strains is contributing to emergence of more XDR TB in the country (57). Identifying primary transmission trends is important in informing prevention programs for drug-resistant TB. Although some aspects are the same when targeting acquired resistance prevention, such as increased diagnostic capabilities, decreased turn-around time, better treatment and follow up, knowledge of primary transmission occurrence increases focus on isolation of drug-resistant TB patients and increased coverage of DST on newly identified TB cases.

Limitations

A limitation of our study is the number of patients enrolled. Although 140 cases of XDR TB were screened for inclusion, only 107 were enrolled. Due to restrictions on isolate availability, 66 of those 107 were used in this analysis. Potential differences in those who were excluded should be considered. Thirty-three patients could not be located, refused consent, or were too ill to participate and did not have a family member to consent for them. These patients may have provided links between more clustered patients, or may have been epidemiologically different from those included in some way. Additionally, some patients were deceased ad the time of the study, information for the social network/epi investigation was given by family members and some contacts/social

congregate locations may have been excluded due to lack of complete knowledge of the patient's social interactions on the part of family members. Survival bias was also of concern in this study. Patients are those with XDR TB in the province who survived long enough to have a DST ordered by a healthcare facility. Given the high mortality rates of those co-infected with HIV, it is likely that those who were most ill did not survive long enough to be included in the study(12, 19).

Implications

This study provides important information on the mechanism for development of XDR TB in KwaZulu Natal; demonstrating that primary transmission may be occurring in a majority of XDR TB cases. Given knowledge of the poor treatment outcomes of XDR TB patients who are co-infected with HIV, and the knowledge of the high prevalence of HIV in South Africa, it is important to develop effective intervention programs focused on the prevention of primary transmission of drug-resistant TB in the province, shifting the focus from prevention of acquired resistance. Knowledge of transmission patterns will help to inform those programs and shape the future of drug-resistant TB prevention.

REFERENCES

1. World Health Organization. Multidrug and extensively drug-resistant TB (M/XDR-TB): 2010 global report on surveillance and response. 2010.
2. World Health Organization. Global tuberculosis report 2012. 2012.
3. World health Organization. Multidrug-resistant tuberculosis (MDR-TB) 2013 Update. 2013.
4. World Health Organization. Guidelines for the programmatic management of drug-resistant tuberculosis. 2011.
5. UNAIDS. UNAIDS World AIDS Day Report. 2011.
6. Chaisson RE, Martinson NA. Tuberculosis in Africa—combating an HIV-driven crisis. *New England Journal of Medicine* 2008;358(11):1089-92.
7. Reid A, Scano F, Getahun H, et al. Towards universal access to HIV prevention, treatment, care, and support: the role of tuberculosis/HIV collaboration. *The Lancet infectious diseases* 2006;6(8):483-95.
8. Daley CL, Small PM, Schecter GF, et al. An outbreak of tuberculosis with accelerated progression among persons infected with the human immunodeficiency virus: an analysis using restriction-fragment—length polymorphisms. *New England Journal of Medicine* 1992;326(4):231-5.
9. Selwyn PA, Hartel D, Lewis VA, et al. A prospective study of the risk of tuberculosis among intravenous drug users with human immunodeficiency virus infection. *The New England journal of medicine* 1989;320(9):545.

10. Antonucci G, Girardi E, Raviglione MC, et al. Risk Factors for Tuberculosis in HIV-lnfected Persons. *JAMA: the journal of the American Medical Association* 1995;274(2):143-8.

11. Wells CD, Cegielski JP, Nelson LJ, et al. HIV infection and multidrug-resistant tuberculosis—the perfect storm. *Journal of Infectious Diseases* 2007;196(Supplement 1):S86-S107.

12. Shah NS, Pratt R, Armstrong L, et al. Extensively drug-resistant tuberculosis in the United States, 1993-2007. *JAMA: the journal of the American Medical Association* 2008;300(18):2153-60.

13. Chideya S, Winston CA, Peloquin CA, et al. Isoniazid, rifampin, ethambutol, and pyrazinamide pharmacokinetics and treatment outcomes among a predominantly HIV-infected cohort of adults with tuberculosis from Botswana. *Clinical infectious diseases* 2009;48(12):1685-94.

14. Vernon A, Burman W, Benator D, et al. Acquired rifamycin monoresistance in patients with HIV-related tuberculosis treated with once-weekly rifapentine and isoniazid. *Lancet* 1999;353(9167):1843-7.

15. Wallengren K, Scano F, Nunn P, et al. Drug-Resistant Tuberculosis, KwaZulu-Natal, South Africa, 2001–2007. *Emerging infectious diseases* 2011;17(10):1913.

16. Kurbatova EV, Taylor A, Gammino VM, et al. Predictors of poor outcomes among patients treated for multidrug-resistant tuberculosis at DOTS-plus projects. *Tuberculosis* 2012.

17. Lawn SD, Harries AD, Meintjes G, et al. Reducing deaths from tuberculosis in antiretroviral treatment programmes in sub-Saharan Africa. *Aids* 2012;26(17):2121-33.

18. Martinson NA, Karstaedt A, Venter WF, et al. Causes of death in hospitalized adults with a premortem diagnosis of tuberculosis: an autopsy study. *Aids* 2007;21(15):2043-50.

19. Gandhi NR, Moll A, Sturm AW, et al. Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. *The Lancet* 2006;368(9547):1575-80.

20. Nelson L, Talbot E, Mwasekaga M, et al. Antituberculosis drug resistance and anonymous HIV surveillance in tuberculosis patients in Botswana, 2002. *The Lancet* 2005;366(9484):488-90.

21. Umubyeyi AN, Vandebriel G, Gasana M, et al. Results of a national survey on drug resistance among pulmonary tuberculosis patients in Rwanda. *The International Journal of Tuberculosis and Lung Disease* 2007;11(2):189-94.

22. Migliori GB, Besozzi G, Girardi E, et al. Clinical and operational value of the extensively drug-resistant tuberculosis definition. *European Respiratory Journal* 2007;30(4):623-6.

23. Mitnick CD, Shin SS, Seung KJ, et al. Comprehensive treatment of extensively drug-resistant tuberculosis. *New England Journal of Medicine* 2008;359(6):563-74.

24. Furin J. The clinical management of drug-resistant tuberculosis. *Current opinion in pulmonary medicine* 2007;13(3):212-7.

25. O'Donnell MR, Padayatchi N, Master I, et al. Improved early results for patients with extensively drug resistant tuberculosis and HIV in South Africa. *The international journal of tuberculosis and lung disease: the official journal of the International Union against Tuberculosis and Lung Disease* 2009;13(7):855.

26. Furin J, Mitnick C, Shin S, et al. Occurrence of serious adverse effects in patients receiving community-based therapy for multidrug-resistant tuberculosis. *The International Journal of Tuberculosis and Lung Disease* 2001;5(7):648-55.

27. Torun T, Gungor G, Ozmen I, et al. Side effects associated with the treatment of multidrug-resistant tuberculosis. *The International Journal of Tuberculosis and Lung Disease* 2005;9(12):1373-7.

28. Brust JC, Shah NS, van der Merwe TL, et al. Adverse Events in an Integrated Home-Based Treatment Program for MDR-TB and HIV in KwaZulu-Natal, South Africa. *JAIDS Journal of Acquired Immune Deficiency Syndromes* 2013;62(4):436-40.

29. World Health Organization. Guidelines for the programmatic management of drug-resistant tuberculosis: emergency update. 2008.

30. Petrini B, Hoffner S. Drug-resistant and multidrug-resistant tubercle bacilli. *International journal of antimicrobial agents* 1999;13(2):93-7.

31. Pillay M, Sturm AW. Evolution of the extensively drug-resistant F15/LAM4/KZN strain of Mycobacterium tuberculosis in KwaZulu-Natal, South Africa. *Clinical infectious diseases* 2007;45(11):1409-14.

32. Victor T, Streicher E, Kewley C, et al. Spread of an emerging Mycobacterium tuberculosis drug-resistant strain in the western Cape of South Africa. *The International Journal of Tuberculosis and Lung Disease* 2007;11(2):195-201.

33. Barnes PF, Cave MD. Molecular epidemiology of tuberculosis. *New England Journal of Medicine* 2003;349(12):1149-56.

34. Alland D, Kalkut GE, Moss AR, et al. Transmission of tuberculosis in New York City--an analysis by DNA fingerprinting and conventional epidemiologic methods. *New England Journal of Medicine* 1994;330(24):1710-6.

35. Frieden TR, Fujiwara PI, Washko RM, et al. Tuberculosis in New York City—turning the tide. *New England Journal of Medicine* 1995;333(4):229-33.

36. Andrews JR, Gandhi NR, Moodley P, et al. Exogenous reinfection as a cause of multidrug-resistant and extensively drug-resistant tuberculosis in rural South Africa. *Journal of Infectious Diseases* 2008;198(11):1582-9.

37. De Jong BC, Antonio M, Awine T, et al. Use of spoligotyping and large sequence polymorphisms to study the population structure of the Mycobacterium tuberculosis complex in a cohort study of consecutive smear-positive tuberculosis cases in The Gambia. *Journal of clinical microbiology* 2009;47(4):994-1001.

38. Pang Y, Zhou Y, Zhao B, et al. Spoligotyping and drug resistance analysis of mycobacterium tuberculosis strains from national survey in China. *PLoS One* 2012;7(3):e32976.

39. Klovdahl A, Graviss E, Yaganehdoost A, et al. Networks and tuberculosis: an undetected community outbreak involving public places. *Social science & medicine* 2001;52(5):681-94.

40. Gardy JL, Johnston JC, Sui SJH, et al. Whole-genome sequencing and social-network analysis of a tuberculosis outbreak. *New England Journal of Medicine* 2011;364(8):730-9.

41. McElroy P, Rothenberg R, Varghese R, et al. A network-informed approach to investigating a tuberculosis outbreak: implications for enhancing contact investigations. *The International Journal of Tuberculosis and Lung Disease* 2003;7(Supplement 3):S486-S93.

42. John E. Multidrug-Resistant Tuberculosis Outbreak among US-bound Hmong Refugees, Thailand, 2005. *Age*;1000(160):58.8.

43. Vella V, Racalbuto V, Guerra R, et al. Household contact investigation of multidrug-resistant and extensively drug-resistant tuberculosis in a high HIV prevalence setting. *The International Journal of Tuberculosis and Lung Disease* 2011;15(9):1170-5.

44. Moonan PK, Quitugua TN, Pogoda JM, et al. Does directly observed therapy (DOT) reduce drug resistant tuberculosis? *BMC public health* 2011;11(1):19.

45. South African Department of Health. The South African Tuberculosis Control Programme practical guidelines. Pretoria: South African Department of Health. 2000.

46. Nardell E, Dharmadhikari A. Turning off the spigot: reducing drug-resistant tuberculosis transmission in resource-limited settings [State of the art series. Drug-resistant tuberculosis. Edited by CY. Chiang. Number 7 in the series]. *The International Journal of Tuberculosis and Lung Disease* 2010;14(10):1233-43.

47. Mitnick C, Bayona J, Palacios E, et al. Community-based therapy for multidrug-resistant tuberculosis in Lima, Peru. *New England Journal of Medicine* 2003;348(2):119-28.

48. World Health Organization. Multidrug and extensively drug-resistant TB (M/XDR-TB): global report on surveillance and response. 2010.

49. World Health Organization. Global tuberculosis report. 2012.

50. DOH. DOH practical guidelines. 2000.

51. Nel A, Mabude Z, Smit J, et al. HIV incidence remains high in KwaZulu-Natal, South Africa: evidence from three districts. *PLoS One* 2012;7(4):e35278.

52. Welz T, Hosegood V, Jaffar S, et al. Continued very high prevalence of HIV infection in rural KwaZulu-Natal, South Africa: a population-based longitudinal study. *Aids* 2007;21(11):1467-72.

53. Small PM, Shafer RW, Hopewell PC, et al. Exogenous reinfection with multidrug-resistant Mycobacterium tuberculosis in patients with advanced HIV infection. *New England Journal of Medicine* 1993;328(16):1137-44.

54. Devaux I, Kremer K, Heersma H, et al. Clusters of multidrug-resistant Mycobacterium tuberculosis cases, Europe. *Emerging infectious diseases* 2009;15(7):1052.

55. Ritacco V, López B, Ambroggi M, et al. HIV infection and geographically bound transmission of drug-resistant tuberculosis, Argentina. *Emerg Infect Dis* 2012;18(11).

56. Small PM, Hopewell PC, Singh SP, et al. The Epidemiology of Tuberculosis in San Francisco--A Population-Based Study Using Conventional and Molecular Methods. *New England Journal of Medicine* 1994;330(24):1703-9.

57. Chihota VN, Müller B, Mlambo CK, et al. Population structure of multi-and extensively drug-resistant Mycobacterium tuberculosis strains in South Africa. *Journal of clinical microbiology* 2012;50(3):995-1002.

TABLES

Table 1. Demographic and clinical characteristics of patients with extensively drug resistant tuberculosis (XDR-TB) (n=66)

Characteristic	n (%)
Age, median (IQR), years*	35 (28-45)
Female sex	36 (55%)
Receipt of previous tuberculosis treatment	42 (64%)
Prior history of multi-drug resistant tuberculosis†	23 (38%)
Prior history of multi-drug resistant tuberculosis: cured/completed tx	5 (22%)
Tested for HIV	66 (100%)
Infected with HIV	50 (76%)
CD4 cell count, median (IQR), cells/mm^3‡	261 (138-434)
Reciept of antiretroviral therapy§	42 (98%)
Any alcohol consumption	16 (24%)
Incarcerated in the past 12 months	1 (1.5%)
Ever worked in a health care facility	3 (4.6%)
Ever worked in a mine	1 (1.5%)
Household member incarcerated in past 12 months	3 (4.6%)
Household member worked in mines in past 12 months	1 (1.5%)

**Age missing on 5 patients*

†Prior history of MDR-TB data missing on 6 patients

‡CD4 count missing on 2 patients with HIV

§Data missing on 7 patients with HIV

Table 2. RFLP patterns for all patients (n=66)

	Number of patients	Number of bands	RFLP pattern(s)
Clustered Patients			
Cluster 1	34	15	HP81
Cluster 2	9	15-17	HP103, HP125, HP126, HP127, HP128, HP129
Cluster 3	3	13-14	MH, MH5
Cluster 4	3	4	AH
Cluster 5	3	8-9	BW657, BW666
Cluster 6	2	11	GY20
Cluster 7	2	13	HP130, HP134
Cluster 8	2	14	HP83
Non-clustered Patients			
Unique 1	1	12	BH70
Unique 2	1	15	HP109
Unique 3	1	14	HP72
Unique 4	1	14	HP72
Unique 5	1	15	HP 81
Unique 6	1	10	KR25
Unique 8	1	0	Mixed
Unique 9	1	18	W151

Table 3. Demographic and clinical characteristics of patients with extensively drug resistant tuberculosis (XDR-TB) (n=66) by cluster status

Characteristic	Cluster (n=58)	Non-cluster (n=8)	p-value
Age, median (IQR), years*	36 (29-46)	31 (28-41)	0.43
Female sex	31 (47%)	5 (63%)	0.72
Receipt of previous tuberculosis treatment	36 (62%)	6 (75%)	0.70
Prior history of multi-drug resistant tuberculosis†	19 (37%)	5 (63%)	0.23
Prior history of multi-drug resistant tuberculosis: cured/completed tx	4 (21%)	1 (20%)	>.99
Infected with HIV	43 (67%)	7 (88%)	0.67
CD4 cell count, median (IQR), cells/mm^3‡	255 (141-353)	522 (135-578)	0.25
Reciept of antiretroviral therapy§	36 (97%)	6 (100%)	>.99
Any alcohol consumption	13 (22%)	3 (38%)	0.39
Incarcerated in the past 12 months	1 (1.7%)	0 (0%)	>.99
Ever worked in a health care facility	1 (1.7%)	2 (25.0%)	0.03
Ever worked in a mine	1 (1.7%)	0 (0%)	>.99
Household member incarcerated in past 12 months	1 (1.7%)	0 (0%)	>.99
Household member worked in mines in past 12 months	1 (1.7%)	0 (0%)	>.99

**Age missing on 5 clustered patients*

†Prior history of MDR-TB data missing on 6 patients

‡CD4 count missing on 2 patients with HIV

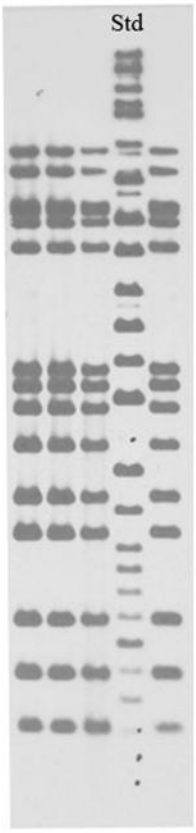
§Data missing on 7 patients with HIV

Table 4. Clustered patients by town of residence (n=19)

Cluster	Patients residing in same city or town	Name of city or town
1	5/34 (15%)	Durban
1	3/34 (9%)	Greytown
1	2/34 (6%)	Ngwavuma
1	3/34 (9%)	Tugela Ferry
1	2/34 (9%)	Verulam
1	2/34 (9%)	Vryheid
2	2/9 (22%)	Pietermaritzburg
6	2/2 (100%)	Durban

FIGURES

Figure 1. Genotyping results for RFLP type HP81



CHAPTER III: SUMMARY, PUBLIC HEALTH IMPLICATIONS, FUTURE DIRECTIONS

In this study, it was concluded that nearly three quarters of cases of XDR TB in the participant population should be attributed to primary transmission. These findings contradict the current focus on acquired resistance as the primary mechanism for development of drug-resistant TB cases. HIV prevalence is high in our study population with over three quarters of participants infected. Prevalence of HIV is also high in this province, with more than 40% of the antenatal population estimated to be infected. Expected outcomes for XDR TB are generally poor regardless of HIV status, but a mortality of almost 100% has been recorded in XDR TB patients who are also co-infected with HIV. This speaks to the urgency of prevention of XDR TB in communities like KwaZulu-Natal.

Current policies in the province do not call for DST on all incident cases of TB. Those patients without prior history of TB are assumed to be drug-susceptible cases and treated empirically as such. Given that almost half of the patients in this study did not have any history of prior tuberculosis treatment, and only one third had any prior history of MDR TB treatment, there is reason to be concerned that drug resistance in the community is higher than indicated by current testing. Patients with active TB disease remain infectious until they have completed 2 months of effective treatment or have a negative smear result. Patients receiving partial or inadequate treatment due to lack of knowledge of their resistance pattern would remain infectious for prolonged periods of time. Moreover, there would be a risk of acquiring further resistance to the 1 or 2 remaining susceptible

medications. Although DST is important in determining current levels of resistance among patients, there may be opposition to its use in all new TB cases due to the expense and availability of resources for testing. However, the cost of generating secondary cases of MDR and XDR TB due to delay in diagnosis of resistance utilizes much of the TB funding for the country. If DST cannot be done on every TB case, use of additional information such as HIV status, potential exposure to DR TB patients, and participation in high-risk activities should be considered when determining whether a patient should receive DST in conjunction with TB treatment history.

This analysis was performed on the initial group of patients enrolled in a study that has a target enrollment approximately 400 XDR TB patients in the province. At the time of analysis, genotyping and sequencing data was available for 66 of the first 107 patients enrolled. There is still information being entered for the patients and more data may be available for them in the future. With the current data, we were unable to identify links in contacts between clustered individuals, as the enrollment numbers grow, we may be able to close those gaps with the addition of reported contacts from future participants. The same is true for data from areas of social congregation and hospitalizations. Despite these limitations, the information from this initial analysis indicates that the larger study will play an integral part in determining the proportion of cases of XDR TB in the province to be attributed each to acquisition and primary transmission. By establishing that a large proportion of cases are the result of primary transmission, effective intervention strategies can be focused on prevention of this pathway to the development of XDR TB.

APPENDICES

APPENDIX I: ADDITIONAL TABLES AND FIGURES

Table 5. CD4 count data from date of sputum collection (n=50)

Days from sputum collection*	Number of patients		Median CD4 (IQR)	
	Clustered	Non-clustered	Clustered	Non-clustered
0-30	12 (32%)	1 (14%)	141 (73-273)	116 (116-116)
31-90	5 (13%)	2 (29%)	225 (76-330)	201 (135-267)
91-180	15 (40%)	3 (43%)	275 (167-413)	547 (522-632)
181-365	6 (16%)	1 (14%)	270 (233-598)	578 (578-578)
Total patients[ǂ]	41/43 (95%)	7/7 (100%)		

**Number of days represents time before or after the date of sputum collection*

ǂDate information missing for 2 clustered patients

Table 6. CD4 count data from date of enrollment (n=50)

Days from enrollment	Number of patients		Median CD4 (IQR)	
	Clustered	Non-clustered	Clustered	Non-clustered
Before enrollment				
0-30	10 (23%)	4 (57%)	266 (233-280)	550 (329-605)
31-90	7 (16%)	2 (29%)	225 (55-291)	332 (116-547)
91-180	10 (23%)	0 (0%)	217 (83-413)	
181-365	4 (9%)	1 (14%)	71 (44-226)	267 (267-267)
After enrollment				
1-30	7 (16%)	0 (0%)	282 (167-454)	
Total patients*[ǂ]	38/43 (88%)	7/7 (100%)		

**Date information missing for 3 clustered patients*

ǂCD4 count date is more than 365 days before enrollment for 2 clustered patients

CD4 counts were compared for clustered and non-clustered patients according to length of time between CD4 count date and date of enrollment and date of sputum collection as illustrated in tables 5 and 6.

Table 7. Genotyping results from selected patients in two clusters (n=5)

Patient	Cluster	RFLP	Bands	katG	rpoB	inhA	gyrA	pncA	rpsL
1	1	HP81	15	AGC(S)315ACC(T)	CTG(L)533CCG(P), GAC(D)516GGC(G)	-8 T > A	AGC(S)95ACC(T), GCG(A)90GTG(V)	insC after 456	AAA(K)121AAG(K)
2	1	HP81	15	AGC(S)315ACC(T)	CTG(L)533CCG(P), GAC(D)516GGC(G)	-8 T > A	AGC(S)95ACC(T), GCG(A)90GTG(V)	insC after 456	AAA(K)121AAG(K)
3	1	HP81	15	AGC(S)315ACC(T)	CTG(L)533CCG(P), GAC(D)516GGC(G)	-8 T > A	AGC(S)95ACC(T), GCG(A)90GTG(V)	insC after 456	AAA(K)121AAG(K)
4	5	BW657	8	AGC(S)315ACC(T)	CAC(H)526TAC(Y)	-15C>T	AGC(S)95ACC(T), GAC(D)94GGC(G)	CAT(H)71TAT(Y)	AAA(K)121AAG(K)
5	5	BW657	8	AGC(S)315ACC(T)	CAC(H)526TAC(Y)	-15C>T	AGC(S)95ACC(T), GCG(A)90GTG(V)	CAT(H)71TAT(Y)	AAA(K)121AAG(K)

Genotyping results in table 7, for selected patients, illustrate the way in which clustering was determined among patients in the study. The three HP81 patients are identical at all resistance-conferring sites and were determined to be clustered. The BW657 patients have one difference at gyrA, but are still considered to be clustered as there are no other differences between them.

Table 8. Places of residence for all clustered patients (n=58)		
Cluster	Number of patients	Place of residence
1	5	Durban
1	3	Greytown
1	3	Tugela Ferry
1	2	Ngwavuma
1	2	Verulan
1	2	Vryheid
1	1	Bergville
1	1	Escourt
1	1	Eshowe
1	1	Glen Anvil
1	1	Harding
1	1	Highflats
1	1	Kwamashu
1	1	Ladysmith
1	1	Manguzi
1	1	Mpangeni Mningi
1	1	Mpumalanga
1	1	Msinga
1	1	Nquthu
1	1	Ozwathini Appelsbosch
1	1	Richmond
1	1	Stanger
1	1	Tongaat
2	2	Pietermaritzburg
2	1	Dundee
2	1	Durban
2	1	Empangeni
2	1	Nkoloweweni
2	1	Nongoma
2	1	Stanger
2	1	Tugela Ferry
3	1	Durban
3	1	Harding
3	1	Peitermaritzburg
4	1	KwaMashu
4	1	Durban
4	1	Vryheid
5	1	Dundee
5	1	Peitermaritzburg
5	1	Pongola
6	2	Durban
7	1	Durban
7	1	Stanger
8	1	Molweni
8	1	Peitermaritzburg

Table 9 includes information on geographical residence for all clustered patients.

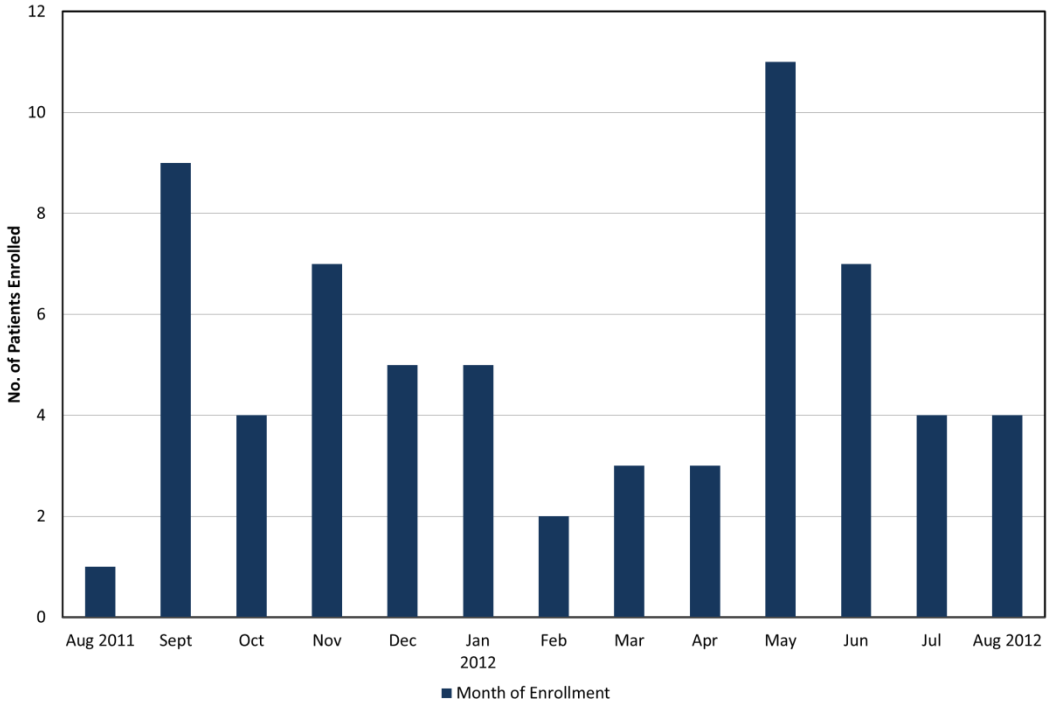


Figure 2. Number of patients enrolled by month, August 2011 to August 2012

APPENDIX II: DATA COLLECTION FORMS

Social Networking Questionnaire (4 pages):

Subject Initials:_____ TRAX Date:__/___/____

Social Network Questionnaire (Page 1 of 4) (DD/MMM/YYYY)

SUBJECT AND INTERVIEW DETAILS

Record with whom this interview is being conducted: (0=Subject; 1=Surrogate)..... ☐

Section 1: LOCATIONS AND CONTACTS AT PLACES OF RESIDENCE (0=No; 1=Yes)

In what year did you first start living at your current residence? .....(YYYY) ____

1.1. Address: _____

1.1.1 Town: _____

Please list all the people who have lived with you at this location in Table 1.0.

2. In the past 5 years, are there any another places, other than the location in Q1, where you have lived or slept for more than 1 month of the year?..... ☐ **If 0=No, skip to Q3**

If YES➔ **Please list the details of the last 5 residences below. Please list all the people who have lived with you at each location in Table 2.0.**

2.1. Address: _____

2.1.1. Town: _____

2.1.2. Approximate dates of residence: (MMM/YYYY) ___/____ to ___/____

2.2. Address: _____

2.2.1. Town: _____

2.2.2. Approximate dates of residence: (MMM/YYYY) ___/____ to ___/____

2.3. Address: _____

2.3.1. Town: _____

2.3.2. Approximate dates of residence: (MMM/YYYY) ___/____ to ___/____

2.4. Address: _____

2.4.1. Town: _____

2.4.2. Approximate dates of residence: (MMM/YYYY) ___/____ to ___/____

2.5. Address: _____

2.5.1. Town: _____

2.5.2. Approximate dates of residence: (MMM/YYYY) ___/____ to ___/____

Subject Initials:_____ TRAX Date:__/___/____

Social Network Questionnaire (Page 2 of 4) (DD/MMM/YYYY)

Section 2: LOCATIONS AND CONTACTS AT PLACES OF WORK (0=No; 1=Yes)

3. Have you ever been employed indoors or in an enclosed area?..... ☐ **If 0=No, skip to Q5**

If YES➔ 3.1. Employer Name *(current or most recent)* / : _____

3.1.1. Address:_____

3.1.2. Town:_____

3.1.3. Approximate dates of employment (MMM/YYYY): ___/____ to ___/____

If still employed, record end date as 333/3333

Please list up to 5 people with whom you have worked most closely at this location in Table 3.0.

4. Have you had any other employment indoors or in enclosed areas in the past 5 years?..... ☐ **If 0=No, skip to Q5**

If YES➔ **Please list up the 5 most recent employers with whom you have worked with below.**

4.1. Employer Name: _____

4.1.1. Address:_____

4.1.2. Town:_____

4.1.3. Approximate dates of employment (MMM/YYYY): ___/____ to ___/____

4.2. Employer Name: _____

4.2.1. Address:_____

4.2.2. Town:_____

4.2.3. Approximate dates of employment (MMM/YYYY): ___/____ to ___/____

4.3. Employer Name: _____

4.3.1. Address:_____

4.3.2. Town:_____

4.3.3. Approximate dates of employment (MMM/YYYY): ___/____ to ___/____

4.4. Employer Name: _____

4.4.1. Address:_____

4.4.2. Town:_____

4.4.3. Approximate dates of employment (MMM/YYYY): ___/____ to ___/____

4.5. Employer Name: _____

4.5.1. Address:_____

4.5.2. Town:_____

4.5.3. Approximate dates of employment (MMM/YYYY): ___/____ to ___/____

Please list up to 5 people with whom you have worked with most closely at each of these locations in Table 4.0.

Subject Initials:_____ TRAX Date:__/___/____

Social Network Questionnaire (Page 3 of 4) (DD/MMM/YYYY)

Section 3: LOCATIONS AND CONTACTS AT PLACES OF SCHOOL (0=No; 1=Yes)

5. Have you attended school in the past 5 years?..... ☐ **If 0=No, skip to Q6**

If YES➔ **Please list up to the 5 most recent school that you have attended below.**

5.1. School Name: _____

5.1.1. Address (*campus, if applicable*):_____

5.1.2. Town:_____

5.1.3 Years of attendance (YYYY): ____ to ____ *If still attending, record end year as 3333*

5.2. School Name: _____

5.2.1. Address (*campus, if applicable*):_____

5.2.2. Town:_____

5.2.3 Years of attendance (YYYY): ____ to ____

5.3. School Name: _____

5.3.1. Address (*campus, if applicable*):_____

5.3.2. Town:_____

5.3.3 Years of attendance (YYYY): ____ to ____

5.4. School Name: _____

5.4.1. Address (*campus, if applicable*):_____

5.4.2. Town:_____

5.4.3 Years of attendance (YYYY): ____ to ____

5.6. School Name: _____

5.6.1. Address (*campus, if applicable*):_____

5.6.2. Town:_____

5.6.3 Years of attendance (YYYY): ____ to ____

Section 4: LOCATIONS AT HOSPITALS (0=No; 1=Yes)

6. Is the subject currently hospitalized at a hospital in KwaZulu-Natal?..... ☐ **If 0=No, skip to Q7**

If YES➔ **Please list details in Table 5.0.**

7. Have you been admitted to a hospital in KwaZulu-Natal in the past 5 years?..... ☐ **If 0=No, skip to Q8**

If YES➔ **Please continue to list all the hospitalizations in the past 5 years in Table 5.0.**

Subject Initials:_____ TRAX Date:__/___/____

Social Network Questionnaire (Page 4 of 4) (DD/MMM/YYYY)

Section 5: OTHER CONGREGATE LOCATIONS AND CONTACTS (0=No; 1=Yes)

8. In the past 5 years, have you been admitted to or stayed overnight in any of the following places:
   - 8.1. Old age home ☐
   - 8.2. Rehabilitation center for alcohol and/or drug abuse ☐
   - 8.3. Jail/prison ☐
   - 8.4. Hostel ☐
   - 8.5. Shelter ☐
   - 8.6. Hospice ☐
   - 8.7. Orphanage ☐
   - 8.8. Mental health residential facility ☐
   - 8.9. Other group living arrangement ☐

If YES to Q8.1.-8.9➔ **Please complete Table 6.0.**

9. In the past 5 years, have you spent time on a regular basis (*at least 2 hours per week*) at any of the following places:
   - 9.1. Bar/shebeen ☐
   - 9.2. Nightclub/discotheque ☐
   - 9.3. Gambling facilities ☐
   - 9.4. Hair salon/barber shop ☐
   - 9.5. Daycare centre/community centre ☐
   - 9.6. Restaurant ☐
   - 9.7. Church or other place of worship ☐
   - 9.8. Gym/workout facility ☐
   - 9.9. Cinema ☐

If YES to Q9.1.-9.9.➔ **Please complete Table 7.0**

10. In the past 12 months, have you regularly commuted or traveled for more than 1 hour per day in the past 12 months? (0=No; 1=Yes) ☐

If YES➔ 10.1. What mode of transportation do you use? (Enter for each 0=No; 1=Yes)
   - 10.1.1. Combi/Taxi ☐
   - 10.1.2. Bus ☐
   - 10.1.3. Car/Bakkie (closed) ☐
   - 10.1.4. Train ☐
   - 10.1.5. Other ☐
     - 10.1.5.1. If 1=YES, specify: _____

11. Are there any other **PLACES** not reported previously where you regularly (at least 2 hours per week) spend time with people indoors in the last 12 months? (0=No; 1=Yes).. ☐

If YES➔ **Please all other locations in Table 8.0.**

12. Are there any other **PEOPLE** not reported previously with whom you regularly (at least 2 hours per week) spend time with indoors in the last 12 months? (0=No; 1=Yes).. ☐

If YES➔ **Please list all other people in Table 9.0.**

Patient Interview Form (4 pages):

Enrollment No:__ __ __ __ __ __ TRAX Date:__ __ / __ __ __ / __ __ __ __

Patient Interview Form (Page 1 of 4) (DD/MMM/YYYY)

Interviewer: Please indicate with whom this interview is being conducted..... ☐
(0=Subject, 1=Surrogate)

Section 1: SOCIODEMOGRAPHIC INFORMATION

1. Subject's gender (0=Male; 1=Female)..... ☐
2. What is your current marital status? ..... ☐
   (0=Single; 1=Cohabiting/Married (Lobola or Civil); 2=Divorced; 3=Separated; 4=Widowed; 8=Don't know)
3. Do you have any children? (0=No; 1=Yes) ..... ☐
   If YES➔ 3.1. How many children you have currently?..... ☐☐
4. What is your total household income per month (Rand)?..... ☐
   (0= Under 500; 1= 500-2499; 2=2500-5000; 3=More than 5000)
   4.1. How many total household members are supported by this income? .... ☐☐
5. Do you receive social grants? (0=No; 1=Yes) ..... ☐
6. Does your household have electricity? (0=No; 1=Yes) ..... ☐
7. Does your household have a flush toilet? (0=No, 1=Yes) ..... ☐
8. In the past 2 years, have you ever lived in the streets? (0=No; 1=Yes)..... ☐
9. What is the highest educational level you have completed?:..... ☐
   (0=No formal schooling; 1=Primary school; 2=Secondary school, but no Matric; 3=Matric;
   4=University or other higher; 8=Don't know)

Section 2: SUBSTANCE USE (0=No; 1=Yes; 8=Don't know)

10. Have you used Dagga (cannabis) in the past year?..... ☐
    If YES➔ 10.1. Did you smoke Dagga?..... ☐
    10.2. Did you use or smoke Dagga with other people?..... ☐
11. Have you used Tik (crystal meth) in the past year?..... ☐
    If YES➔ 11.1. Did you smoke Tik?..... ☐
    11.2. Did you use or smoke Tik with other people?..... ☐
12. Have you used Mandrax (Quaaludes) in the past year?..... ☐
    If YES➔ 12.1. Did you smoke Mandrax?..... ☐
    12.2. Did you use or smoke Mandrax with other people?..... ☐
13. Have you used Whoonga (efavirenz) in the past year?..... ☐
    If YES➔ 13.1. Did you smoke Whoonga?..... ☐
    13.2. Did you use or smoke Whoonga with other people?..... ☐
14. Have you used Heroin in the past year?..... ☐
    If YES➔ 14.1. Did you smoke Heroin?..... ☐
    14.2. Did you use or smoke Heroin with other people?..... ☐
15. Have you used Cocaine in the past year?..... ☐
    If YES➔ 15.1. Did you smoke Cocaine?..... ☐
    15.2. Did you use or smoke Cocaine with other people?..... ☐

Enrollment No:__ __ __ __ __ TRAX Date:__ __ / __ __ __ / __ __ __ __

Patient Interview Form (Page 2 of 4) (DD/MMM/YYYY)

Section 3 determines the current and past smoking history (use of tobacco products other than dagga) of subjects.
Please use the following guide:
- Rare instances of smoking or experimental smoking (tried once or twice in lifetime) should be counted in the NO category

Section 3: SMOKING HISTORY (USE OF TOBACCO PRODUCTS OTHER THAN DAGGA)

16. Do you currently smoke? ☐
(0=No; 1=Yes; 8=Don't know)

If YES➔ 16.1. How many cigarettes do you smoke each day? ☐☐

16.2. How many years ago did you start smoking? ☐☐

16.3. Do you smoke indoors with other people? (0=No; 1=Yes; 8=Don't know) ☐

16.4. Do you share hand-rolled blunts, pipes, water pipes or other smoking aides with others? (0=No; 1=Yes; 8=Don't know) ☐

17. In the past, have you smoked tobacco? ☐
(0=No; 1=Yes; 8=Don't know)

Section 4 refers to the number of alcoholic "drinks" a subject consumes.
Please use the following guide:
- 1 drink = 1 can / bottle of beer / wine cooler
- 1 drink = 1 glass of wine
- 1 drink = 1 "tot" of liquor
- 1 drink = 1 mixed drink made with 1 shot of liquor

Section 4: ALCOHOL USE

18. How often have you had a drink containing alcohol in the past year? ☐ **If 0=NEVER, skip to Q26.**
(0= Never; 1= Less than once a month; 2= 2 to 4 times a month;
3= 2 to 3 times a week; 4= 4 or more times a week; 8=Don't know)

19. In the past year, how many alcoholic drinks do you have on a typical day when you are drinking? ☐
(0= 1-2 drinks; 1= 3-4 drinks; 2= 5-6 drinks; 3= 7-9 drinks; 4= 10 or more drinks; 8=Don't know)

20. In the past year, how often do you have six or more drinks on one occasion? ☐
(0= Never; 1= Less than once a month; 2= Monthly; 3= Weekly; 4= Daily or almost daily; 8=Don't know)

If <u>BOTH</u> Q19=0 AND Q20=0, skip to Q26.

21. How often, during the last year, have you found that you were not able to stop drinking once you had started? ☐
(0= Never; 1= Less than once a month; 2= Monthly; 3=Weekly; 4= Daily or almost daily, 8=Don't know)

22. How often during the last year have you failed to do what was normally expected of you because of drinking? ☐
(0=Never; 1=Less than once a month; 2=Monthly; 3=Weekly; 4=Daily or almost daily; 8=Don't know)

23. How often, during the last year, have you needed a first drink in the morning to get yourself going after a heavy drinking session? ☐
(0= Never; 1= Less than once a month; 2= Monthly; 3=Weekly; 4= Daily or almost daily; 8=Don't know)

24. How often, during the last year, have you had a feeling of guilt or remorse after drinking? ☐
(0= Never; 1= Less than once a month; 2= Monthly; 3=Weekly; 4= Daily or almost daily; 8=Don't know)

Enrollment No:__ __ __ __ __ TRAX Date:__ __ / __ __ __ / __ __ __ __

Patient Interview Form (Page 3 of 4) (DD/MMM/YYYY)

Section 4 (cont): ALCOHOL USE

25. How often during the last year have you been unable to remember what happened the night before because you had been drinking? ☐
(0= Never; 1= Less than once a month; 2= Monthly; 3= Weekly; 4= Daily or almost daily, 8=Don't know)
26. Have you or someone else ever been injured as a result of your drinking? ☐
(0= No; 2=Yes, but not in the last year; 4=Yes, during the last year; 8=Don't know)
27. Has a relative or friend or a doctor or another health worker ever been concerned about your drinking or suggested you cut down (not as a result of TB)? ☐
(0= No; 2=Yes, but not in the last year; 4=Yes, during the last year; 8=Don't know)

Section 5: TB SYMPTOMS AT XDR TB DIAGNOSIS

28. In the last 6 months, have you had a cough? (0=No; 1=Yes; 8=Don't know) ☐
If YES➔ 28.1. Is / Was the cough productive? (0=No; 1=Yes; 8=Don't know) ☐
28.2. When did the cough begin? (MMM/YYYY) __ __ __ / __ __ __ __
28.3. How many weeks did you have the cough? ☐☐
(If more than 2 years, record 99)

Section 6: TB EXPOSURE HISTORY (0=No; 1=Yes; 8=Don't know; 9=Not Applicable)

29. Have you been previously treated for TB? ☐
If YES➔ 29.1. What kind of treatment did you receive? ☐
(1=1st line treatment; 2=1st line treatment with Streptomycin; 3=MDR treatment, 8=Don't know)
30. Have you ever been treated for TB with injections? ☐
31. Have you been in prison in the past 12 months? ☐
32. Are you currently or were you ever working in a health care facility? ☐
33. Do you currently or did you ever work in a mine? ☐
34. Has any household member been in the mines in the past 12 months? ☐
35. Has any household member been in prison in the past 12 months? ☐
If YES ➔ 35.1. When did this household member come out of prison? __ __ / __ __ __ / __ __ __ __

Section 7: PAST MEDICAL HISTORY (0=No; 1=Yes; 8=Don't know)

36. Were you treated at the following TB specialty hospitals in the past 5 years:
(Enter for each location 0=No; 1=Yes; 8=Don't know)
36.1. King George V Hospital ☐
36.2. Greytown Hospital ☐
36.3. Murchison Hospital ☐
36.4. Thulasizwe Hospital ☐
36.5. Manguzi Hospital ☐
36.6. Catherine Booth-Amathikhulu ☐
36.7. Doris Goodwin –Edendale PMB ☐
36.8. Other ☐
36.8.1. If 1=YES, specify: _____

Enrollment No:__ __ __ __ __ TRAX Date:__ __/ __ __ __/ __ __ __ __
Patient Interview Form (Page 4 of 4) (DD/MMM/YYYY)

Section 7 (cont): PAST MEDICAL HISTORY (0=No; 1=Yes; 8=Don't know)

37. Have you ever been diagnosed with Diabetes? ☐

If YES➔ 37.1. When were you diagnosed with Diabetes? __ __ __/__ __ __ __
(MMM) (YYYY)

37.2. Are you taking insulin treatment for your Diabetes? ☐

Section 8: HIV DIAGNOSIS AND HISTORY

38. Have you ever been tested for HIV? (0=No; 1=Yes; 8=Don't know) ☐ **If 0=No OR 8=Don't know skip to Q42**

If YES➔ 38.1 What is your HIV status? (0=Negative; 1=Positive; 8=Don't know) ☐

39. If patient is HIV-negative, have you been tested in last 3months? ☐ **If 0=No, skip to Q42**
(0=No; 1=Yes; 9=Not Applicable, because patient is HIV-positive)

If YES➔ 39.1. What was the date of the test? (MMM/YYYY) __ __ __/__ __ __ __

40. If patient is HIV-positive, are you currently on ARVs? ☐
(0=No; 1=Yes; 8=Don't know; 9=Not Applicable, because patient is HIV-negative)

If YES➔ 40.1. ARV start date? (MMM/YYYY) __ __ __/__ __ __ __

41. If patient is HIV-positive, what is your most recent CD4 count? ☐☐☐☐ cells/mm^3
(9999=Not Available)

41.1. Date of test (DD/MMM/YYYY) __ __/__ __ __/__ __ __ __

Section 9: HIV TESTING AND SPECIMEN COLLECTION

Offer HIV testing if:
- Patient reports s/he is HIV-negative but last test was more than 3 months ago
- Patient reports s/he is HIV-negative, but no documentation is provided

If patient is 2 years or younger, refer to local clinic for paediatric HIV testing.

42. Has the subject consented to have HIV testing? ☐
(0=No; 1=Yes; 9=Not Applicable, patient is 2 years or younger)

If YES➔ 42.1. What is the result of the HIV test? ☐
(0=Negative; 1=Positive; 2=Indeterminate)

43. Was blood collected? (0=No; 1=Yes; 9=Not Applicable) ☐

44. Was a sputum sample collected? (0=No; 1=Yes; 9=Not Applicable) ☐

45. Did the subject consent for future storage? ☐
(0=No; 1=Yes; 9=Not Applicable, because patient is deceased or did not consent)

APPENDIX III: ETHICAL APPROVAL

EMORY UNIVERSITY | Institutional Review Board

TO: Neel Gandhi, PhD
Principal Investigator
Epidemiology

DATE: October 9, 2012

RE: **Expedited Approval**
IRB00060394
Transmission of HIV-Associated XDR TB in Rural South Africa

Thank you for submitting a new application for this protocol. This research is eligible for expedited review under 45 CFR.46.110 and/or 21 CFR 56.110 because it poses minimal risk and fits the regulatory categories F(2), (3), (5), and (7) as set forth in the Federal Register. The Emory IRB reviewed it by expedited process on 10/7/2012 and granted approval effective from **10/7/2012** through **10/6/2013**. Thereafter, continuation of human subjects research activities requires the submission of a renewal application, which must be reviewed and approved by the IRB prior to the expiration date noted above.

- Approved under 45 CFR 46.404 as research that poses no more than minimal risk to pediatric subjects
- One parent's signature is sufficient in providing the of consent of a minor
- The following protocol and consent documents are approved for use in association with this study:
  - TRAX Protocol v1.0, version date 5/19/2011
  - Adult Consent form v6.0 (English), version date 8/3/2012
  - Parent Consent form v6.0 (English), version date 8/3/2012
  - HIV Consent form v3a (English), version date 2/24/2010
  - HIV Consent form v3a (Zulu), version date 2/24/2010
  - Assent form v6.0 (English), version date 8/3/2012
  - Assent form v6.0 (Zulu), version date 8/3/2012

Any reportable events (e.g., unanticipated problems involving risk to subjects or others, noncompliance, breaches of confidentiality, HIPAA violations, protocol deviations) must be reported to the IRB according to our Policies & Procedures at www.irb.emory.edu, immediately, promptly, or periodically. Be sure to check the reporting guidance and contact us if you have questions. Terms and conditions of sponsors, if any, also apply to reporting.

Before implementing any change to this protocol (including but not limited to sample size, informed consent, study design, you must submit an amendment request and secure IRB approval.

In future correspondence about this matter, please refer to the IRB file ID, name of the Principal Investigator, and study title. Thank you

Sam Roberts, CIP
Research Protocol Analyst
This letter has been digitally signed