The Transmission of *Mycobacterium tuberculosis* in High Burden Settings

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Unacceptable levels of Mycobacterium tuberculosis transmission are observed in high burden settings and a renewed focus on cutting person-to-person transmission in these communities is needed. Here, we review recent developments in our understanding of airborne transmission. We outline approaches to measuring transmission in populations and trials and we describe the Wells-Riley Equation, which is used to estimate transmission risk in indoor spaces. Current research priorities include identifying effective tuberculosis infection control strategies; better understanding where transmission occurs and the transmissibility of drug resistant strains; and estimating the impact of HIV and antiretroviral therapy on transmission dynamics. When planning research and designing interventions to interrupt transmission, resource constraints common in high burden settings, including shortages of healthcare workers, must be considered.

Introduction

Sustained declines in disease incidence of up to 20% per year are required to meet the targets set out in the World Health Organization (WHO) End-TB Strategy.^{1, 2} However, incidence is only estimated to be declining at 1.5% per annum.³ This trend is consistent with model predictions regarding the likely impact of current control strategies,⁴ which focus on case detection and treatment completion.⁵ Even in areas with good rates of case finding and treatment completion, evidence suggests that transmission remains a problem. Although quality data on active tuberculosis (TB) in children under the age of 5 years are limited, paediatric cases indicate continuing high levels of transmission.^{3, 6, 7} Recent tuberculin surveys in high-prevalence countries estimate annual risks of *Mycobacterium tuberculosis* (MTB) infection of 0.3-2.2%,⁸⁻¹² but exceeding five per cent in some parts of southern Africa.^{13, 14} Test reversions (negative tests in people who previously had a positive test) mean such cross sectional surveys may underestimate transmission.¹⁵ Data on MTB transmission derived using molecular typing methods from high burden areas are limited to a small number of research active settings. Nevertheless, these data suggest more disease results from recent transmission than from reactivation of latent TB,^{16, 17} particularly in HIV positive people.¹⁸ The rapid rebound in TB incidence following the discontinuation of isoniazid preventive treatment (IPT) in recent Southern African studies suggest on-going transmission remains important in high burden settings,^{19, 20} although models predict a contribution from reactivation disease implying IPT may not sterilise.^{21, 22}

To achieve the goals of the End-TB strategy,² an increased emphasis on reducing person-to-person MTB transmission in high burden settings is needed. This review summarises recent research on MTB transmission in these settings. We focus on the biology of airborne MTB transmission, measuring transmission in populations, and modelling transmission using the Wells-Riley approach. We conclude by identifying research priorities. We do not discuss transmission-blocking vaccines nor mixed infections, each the subject of a recent review article.^{23, 24} It is important to note that there is no international consensus on TB incidence or prevalence thresholds that constitute 'high burden', although a TB incidence of 100 per 100,000 per year has recently been used by the WHO.²⁵ Most of the studies we review were conducted in communities with a TB incidence of 100 per 100,000 per year or more.

Airborne MTB transmission

Whilst MTB complex organisms can be spread via unpasteurised milk, direct inoculation and other means, we focus on the predominant route, airborne transmission. The fundamentals of airborne MTB transmission were described by William Frith Wells, Richard Riley, Robert Loudon, Rena Roberts, and others, more than sixty years ago.²⁶ Although much remains unknown, progress in basic and clinical sciences has improved our understanding of MTB transmission that, until recently, had remained largely unchanged for over 50 years. Figure 1 illustrates the MTB transmission cycle. Interrupting any process in MTB's natural history will reduce rates of transmission at a population level.

Aerosol generation and inhalation: Individuals with pulmonary TB aerosolise MTB, placing their contacts at risk of infection (Figure 1). This occurs at a faster rate during coughing.²⁷ There is some evidence that speech²⁸ and singing²⁹ are effective aerosol generating activities, though these studies focussed only on droplets originating in the mouth. Whilst the largest respiratory droplets fall to the ground, rapid evaporation means many droplets attain a sufficiently low mass before settling that they remain suspended in air currents until either inhaled or ventilated out of the room.³⁰ Important new insights into MTB transmission have come from 'cough box' experiments.³¹ Here, TB patients were asked to cough 'as frequently as was comfortable' for five minutes into a 'cough aerosol sampling system'. Whilst this may not be physiological, these experiments suggest that most MTB that is aerosolised during coughing is in droplets small enough, even without evaporation, to remain suspended in the air.³¹ These cough box experiments^{31, 32}, consistent with studies from guinea pig facilities^{33, 34} and molecular epidemiological observations³⁵⁻³⁷, suggest that some people with TB may be much more infectious than others. Early animal

experiments demonstrated that MTB in smaller droplets more readily produced tubercles in the lung than did MTB in larger droplets, presumably as they escape filtration in the upper airways.³⁸ The use of surgical masks by TB patients has been shown to reduce transmission to guinea pigs by 56%, suggesting they partially block aerosolisation of the relevant respiratory droplets.³⁹

Establishing infection or disease: The quantity and characteristics of the inhaled droplet predict clinical outcomes, with early experiments demonstrating that infectious dose predicts risk of progression to disease.⁴⁰ In the cough box experiments, the quantity of aerosolised MTB produced by individuals predicted infection in household contacts better than smear grade or time to culture positivity.³² It has recently been proposed that larger droplets, settling in the upper airway, might result in immune memory and a positive test for infection but little risk of progression to disease.⁴⁰ That some highly exposed individuals fail to become TST or IGRA positive and the recent discovery of genetic loci that predict TST positivity suggest that it is possible to clear MTB infection without developing an adaptive immune response – so called ‘early clearance’.⁴¹ This process is probably important epidemiologically but, given it leaves no footprint, is difficult to study. There is a growing appreciation, informed by animal studies and advanced imaging techniques, that a binary classification of TB into latent infection and active disease may be too simplistic.^{42, 43} Some individuals with positive tests for infection may have cleared the organism,⁴³ and periods of active replication in ‘latent TB’ have been observed.⁴² Pulmonary MTB infection towards the more active end of the infection-to-disease spectrum is probably necessary for infectiousness.

Duration of infectiousness: There is a widely-held view that the infectiousness of patients diminishes sufficiently after two weeks of anti-tuberculous therapy such that transmission to contacts is unlikely.⁴⁴ Many guidelines rely on proxy measures of infectiousness, e.g. smear status or culture conversion times. It is worth noting that median time to culture conversion amongst patients treated under daily direct observation for drug-susceptible TB in Peru was 37 days.⁴⁵ However, in patients on treatment, the association between sputum smear or culture status and infectiousness is not straightforward,⁴⁶⁻⁵⁰ and is likely to be influenced not only by organism viability in respiratory secretions but also by the capacity to generate aerosolised MTB through coughing.²⁷ Since cough frequency diminishes on treatment,⁵¹ assumptions about infectiousness based upon culture conversion times may overestimate risk. Furthermore, organisms that propagate in culture may not thrive when exposed to a hostile immune system in the alveoli.

Infectiousness can be studied in guinea pig facilities in which the number of animals infected following exposure to air exhausted from isolation rooms containing TB patients is measured. Such experiments show that effective therapy is associated with markedly fewer MTB infections than are seen before treatment is initiated or when isolates are not fully susceptible to the treatment regimen.^{34, 50} However, the guinea pigs in these experiments remained exposed to the patients for many weeks. Thus, these experiments have not yet reliably established a time window after which a patient can be considered to be no longer infectious. Most, if not all, household contacts are more likely to be infected by an index patient in the pre-treatment period than once treatment is initiated.⁵² This is due to both the likely longer duration of exposure and to greater infectiousness pre-treatment. This may also hold for patients with drug-resistant TB, amongst whom culture conversion times will typically be longer, particularly if there is delay in initiating effective treatment.

Measuring transmission in populations

Even in the highest burden communities, the prevalence and annual incidence of active TB disease rarely exceed 2%. This, in addition to incomplete surveillance data, particularly in high burden settings, and tests for MTB infection with poor sensitivity and specificity, make the measurement of MTB transmission in populations a challenge and the routine determination of incident TB disease unfeasible.⁵³ Measuring

transmission therefore relies on proxy measures, assumptions and combination approaches. There is debate about the best approach to measuring MTB transmission in trials of control interventions.⁵⁴⁻⁵⁶ Short-term reductions in disease prevalence, for example, are difficult to interpret as prevalence is influenced not only by transmission, but also by progression from infection to disease and disease duration.⁵⁴ The common tests for MTB infection are summarised in Panel 1. Approaches to measuring MTB transmission in populations are summarised in Table 1.

Traditional approaches: Tuberculin surveys in school children are a classical means of estimating MTB transmission at a population level. While age assortative mixing may mean paediatric infections do not fully reflect MTB transmission between adults^{54, 57, 66}, repeated TST surveys might still allow estimates of the trend in force of infection over time to be attained. Trend estimates based on tuberculin surveys are fairly robust and not greatly influenced by the proportion of children with BCG scars or the cut point used to define a 'positive' test.^{65, 67}

Molecular approaches: Tools for strain typing MTB include spoligotyping, which has limited resolution; Restriction Fragment Length Polymorphism (RFLP)⁶⁸ and Mycobacterial Interspersed Repetitive Units – Variable Number of Tandem Repeats (MIRU-VNTR),^{69, 70} both of which have been widely used; and Whole Genome Sequencing (WGS). These molecular epidemiological tools provide evidence for or against potential linkages between two or more cases of active TB and have led to a number of critical insights into MTB transmission.⁷¹ As these techniques require a bacterial isolate, molecular epidemiology, with rare exceptions,⁷² only captures infections that have progressed to active TB disease.⁷³ Molecular epidemiology cannot distinguish changes in transmission intensity from changes in the rate of progression to active TB shortly after infection resulting, for example, from varying levels of immunosuppression. The better resolution of WGS and steep reductions in cost mean it is likely to eventually replace existing strain typing techniques. However, molecular epidemiology using WGS will require an understanding of the rate at which mutations occur. Recent studies suggest that, in active disease, single nucleotide polymorphisms (SNPs) emerge, on average, at half a SNP per genome per year or slower.^{37, 74-76} Most of the patients in these studies were on TB treatment and there was considerable variation in the rate at which mutations occurred. Primate studies suggest a similar mutation rate and that the mutation rate may not differ substantially between active and latent infection.⁷⁷ However, limited data suggest that, in man, mutations accumulate more slowly during latent infection.⁷⁸ Occasional accelerated intra-patient 'microevolution' events,⁷⁹ and the slow rate at which SNPs accumulate may make inferring chains of transmission from MTB genotypes alone challenging. Probabilistic models that also incorporate epidemiological and clinical data may be needed.⁸⁰ With molecular epidemiology using WGS, as with older strain typing techniques, adequate study duration, a high sampling fraction and careful documentation, follow up and reporting are important.^{16, 81-83} However, novel approaches to the analysis of sequence data may allow population level inferences to be made from smaller samples.

Air sampling: There is interest in attempting to directly detect aerosolised MTB from room air. This might allow quantification of MTB exposure in putative sites of transmission. A few demonstration studies, using polymerase chain reaction (PCR) on room air filtrate, suggest this may be feasible.⁸⁴⁻⁸⁶ Whilst PCR detection of MTB DNA does not necessarily mean organisms are viable, it suggests that individual(s) with pulmonary TB have produced bioaerosols in the space. This should be, at least in theory, a reasonable proxy for transmission risk.

The Wells-Riley Equation

Room ventilation and social contact patterns determine whether others are exposed to MTB that has been aerosolised. The Wells-Riley Equation⁸⁷ is used to model the transmission of respiratory pathogens, such as MTB, that are spread by crowd rather than close contact. Transmission risk in a defined space over time t is modelled as a Poisson process.

$$\text{Probability of transmission} = 1 - e^{-Iqpt/Q}$$

I is the number of infectious individuals present

q is the rate at which infectious individuals produce infectious 'quanta'

p is the rate at which susceptible individuals breathe

and Q is the rate at which air from the space is exchanged with uncontaminated air (ventilation)

Riley and colleagues defined 'quanta' as 'the number of infectious airborne particles required to infect which may be one or more airborne particles'.⁸⁷ This value is often assumed or fitted to data. Various attempts have been made to empirically estimate q for TB by venting air exhaled by TB patients over experimental animals. Two sets of experiments in the pre-HIV era estimated quanta production at 0.62-0.82⁸⁸ and 1.25⁸⁹ per hour using a heterogeneous group of TB patients.^{39, 90} More recently, Escombe *et al.* obtained an estimate of 8.2 quanta per hour in a group of HIV positive patients in Lima, Peru.³⁴ These data disguise huge heterogeneity in infectiousness with the most infectious patients in each study producing 60 and 226 infectious quanta per hour respectively. High rates of quanta production have been measured in patients with advanced multidrug-resistant TB (MDR-TB),³⁹ and very high rates estimated in outbreaks related to aerosol generating procedures.⁹¹ Interestingly, estimates of q obtained by fitting to data from high burden communities are lower than those obtained empirically.⁹² This may be because untreated patients in the community are at an earlier stage in their illness, and hence less infectious than the diagnosed patients used in the animal studies.

The Wells-Riley Equation has a number of important limitations. It assumes that air in the space is fully mixed, and does not account for heterogeneity in infectiousness or susceptibility to infection. Adaptations to the equation have been published. One widely used variant uses instead a 'rebreathed fraction' obtained from paired indoor and outdoor carbon dioxide (CO₂) measurements.⁹³ This avoids the need to measure Q , which can be technically challenging. In many settings, there are substantial spatial and temporal variations in CO₂ concentrations. Not obtaining contemporaneous CO₂ measurements from directly outside the buildings studied may be reasonable where strong winds limit local spatial and temporal variations in CO₂.^{92, 94} but would not be reasonable in other circumstances. A number of important insights have been derived using the Wells-Riley approach. For example, one study suggested that active case finding could not control high levels of MTB transmission in a South African prison if levels of overcrowding and poor ventilation were not also addressed.⁹⁵ Another South African paper, which used the equation to predict settings in which MTB transmission might occur, is described below.⁹²

Research Priorities

There is much left to be learned about MTB transmission. Here, we outline priority areas for future research. Approaches to interrupting MTB transmission include active case finding, the provision of isoniazid preventive therapy (IPT) and TB infection control. There have recently been large trials published of active case finding and mass IPT to interrupt MTB transmission. The ZAMSTAR result may be the first empirical data suggesting that active screening for TB disease impacts on TB transmission at a population-level.^{55, 96} The Thibela TB trial, conducted in a setting with a very high force of infection, found mass administration of IPT protected individuals whilst on treatment but had no impact on TB incidence in the wider community.^{20, 22}

Infection control: There has been much less research on TB infection control, which is conventionally described in three domains – administrative controls (which aim to minimise contamination of shared air by infectious subjects e.g. cough triage, early diagnosis and treatment), environmental controls (which aim

to minimise exposure through removal of contaminated air) and personal protection measures (which aim to minimise inhalation of contaminated air e.g. N95 respirators).⁹⁷ A review of observational and animal studies concluded that there is strong evidence supporting the role of ventilation as an environmental control in reducing the risk of airborne transmission of MTB.⁹⁸ Of the many ways of increasing ventilation, increased mechanical ventilation,⁹⁹ natural ventilation through increased window-opening,¹⁰⁰ and wind-driven roof turbines have been considered specifically as means to reduce MTB infection risk.¹⁰¹ Natural ventilation has been recommended by the WHO as an effective way to reduce infection.⁹⁷ Air disinfection tools, particularly upper room Ultra Violet Germicidal Irradiation (UVGI), have also been studied with UVGI leading to steep reductions in MTB transmission from TB patients to experimental animals^{102, 103}

Implementing environmental controls is not always straightforward. An increase in indoor levels of outdoor pollution, security concerns, exposure to outdoor hazards such as disease vectors, a loss of thermal comfort, energy loss through the exfiltration of conditioned (heated or cooled) indoor air, and higher running and maintenance costs of mechanical systems are side-effects of increased ventilation,¹⁰⁴ and may make such measures unacceptable to occupants. Therefore, the ideal retrofit and design measures employed in a building should account for occupant patterns, numbers, and preferences, the climate and surrounding environment, building geometry, and the materials and budget available. Building simulation tools may be employed to predict the optimal design or retrofit of buildings to maximise ventilation according to specified criteria.^{105, 106}

The FAST approach to TB infection control in congregate settings has recently been promoted and advocates Finding TB cases Actively, Separating safely, and Treating effectively.¹⁰⁷ A trial of the FAST approach is about to commence in Peru (NCT 02355223) with TST conversion among healthcare workers as an endpoint. However, the lack of a comparator group may limit the strength of the conclusions that can be reached. While a relationship between ventilation rate and TB transmission is clear, there is little empirical evidence on the effectiveness of infection control interventions in reducing transmission, with the majority of studies using animal surrogates or ventilation measurements as a proxy for transmission risk. A notable exception was the Tuberculosis Ultraviolet Shelter Study, which showed that environmental modifications can be safely implemented at scale.¹⁰⁸ Whilst there were too few TST conversions among residents of the shelters to demonstrate an impact on MTB transmission, similar studies in higher burden settings would be valuable in quantifying the impact of TB infection control interventions on transmission to human occupants and, potentially, on transmission in the surrounding community.¹⁰⁹ An important knowledge gap is whether domestic infection control implemented at diagnosis can mitigate against secondary infections in patients managed in the community. To our knowledge, there have been no trials of such interventions. This is an important question, perhaps in MDR-TB and certainly in extensively drug-resistant TB (XDR-TB), where chemotherapy may not promptly reduce infectiousness and where the consequences of transmission may be severe.

Locating MTB transmission: Historically, households have been considered a major focus of MTB transmission. However, three molecular epidemiology studies from Sub Saharan Africa,^{17, 110, 111} and one from rural Vietnam,¹¹² all suggest that most transmission occurs between rather than within households. In these high burden settings, this likely reflects a high transmission risk outside the home rather than a reduction in the risk of transmission to household contacts. Other evidence also suggests that, overall, most transmission may occur outside the household,^{113, 114} but studies suggest this is age dependent with young children more likely to have been infected by a household member.¹¹⁵⁻¹¹⁷ It is likely that transmission in indoor congregate settings is important in high burden settings. For example, time working in public transport is strongly associated with TST positivity in Lima, Peru.¹¹⁸ Understanding which settings are

important should be a research priority, as this would allow infection control and active case finding interventions to be better targeted.¹⁰⁹

The Wells-Riley Equation and its variants have been used to estimate MTB transmission risk by location. Studies adopting these approaches have estimated ventilation or likely exposure to exhaled bioaerosols based on CO₂ levels. These methods have recently been applied to study transmission in a Cape Town township. Data were collected on CO₂ concentrations in multiple settings visited by 63 adolescents. It was estimated that 93% of total exposure to 'rebreathed air' occurred in a few locations: own home, visited homes, public transport, work or school.⁹⁴ The same research group used a modified Wells-Riley Equation, CO₂ measurements and social contact pattern data to estimate the proportion of overall MTB transmission by location. They found that, in the same Cape Town township, 50% of incident infections among 15-19 year olds may take place in school, that workplaces are important places for adult transmission and that household and public transport may be important sites of transmission between age groups.⁹² These inferences are potentially useful but the studies were small. The conclusions may be context-specific and assume TB disease prevalence is the same in each location within an age and sex group, which is probably not the case. Similar studies need to be undertaken on larger scales and in more settings, ideally combined with location specific estimates of TB prevalence.

Health facilities, particularly in HIV endemic areas, are an important setting in which infectious TB patients mix with susceptible persons. TB patients attend healthcare facilities prior to diagnosis, when presenting with TB symptoms, and during the course of TB treatment. Delays in recognition, diagnosis and isolation of infectious cases augment the risk of nosocomial transmission from unsuspected index cases. Overcrowded outpatient clinics and Emergency Departments congregate vulnerable subjects in settings where the likelihood of exposure to patients with infectious TB is relatively high. In one Emergency Department study in Lima, Peru, IGRA conversion was observed in 30% of healthcare workers during a 12 month period compared to 0% of hospital security and domestic personnel;¹¹⁹ remarkably one third of patients identified with TB in the study were attending the hospital for an apparently unrelated reason (trauma, pregnancy etc.) highlighting the importance of the unrecognized risk. Nosocomial transmission played an important role in the Tugela Ferry extensively drug resistant tuberculosis XDR-TB outbreak.¹²⁰ Future research should attempt to quantify the proportion of MTB transmission in high burden settings that occurs within healthcare facilities, as well as the impact of programmatic infection control interventions on this proportion.

Spatial heterogeneity in the incidence of TB and drug-resistant TB is evident in analyses of programmatically collected data and has been well documented in many studies,¹²¹⁻¹²³ raising the possibility that targeted interventions may be effective. At this time, however, the mechanisms driving such heterogeneity are not completely understood; for example, localized transmission and or aggregation of individuals sharing risk factors for infection or progression may combine to generate such patterns of disease. A better understanding of this spatial heterogeneity might usefully inform targeted TB control interventions. The spatial extent of TB transmission networks in these settings is not known. Better data on the geographical extent of social contacts relevant for TB transmission will assist in designing intervention studies and it would be helpful to see research addressing this question.

Drug resistance and transmissibility: Globally, there were an estimated 480,000 incident cases of MDR-TB in 2013. The proportion of new cases that are infected with MDR-TB is around 3.5% and this has not changed appreciably over the period 2008-13.³ MDR-TB is disproportionately distributed, with the highest rates seen in Central Asia and Eastern Europe where, in several countries, a high proportion of MDR-TB cases have no previous history of TB treatment. This suggests high levels of MDR-TB transmission.

Projections of the future burden of MDR-TB depend critically on estimates of the reproductive potential of drug resistant strains as compared to drug sensitive strains.^{124, 125} This reproductive potential, which can be quantified as the expected number of secondary infectious cases attributable to a single infectious case, is the product of several factors: the duration of infectiousness, the rate at which respiratory exposures occur, the probability that exposure results in transmission, and the probability that infection progresses to infectious active disease.¹²⁶ Drug-resistance may affect several of these factors. For example, the duration of infectiousness is often longer in individuals with MDR-TB as delays in the diagnosis of resistance can lead to delayed initiation of effective treatment. However, the probability of establishing a successful infection or of infection leading to disease will be decreased if resistance-conferring mutations have a fitness cost.¹²⁷ *In vitro* experiments (e.g. competitive growth assays which may measure biological fitness) and observational studies (e.g. contact tracing and molecular clustering studies which measure effects of both biological fitness and differences in duration of infectiousness) suggest a wide variety of possible overall relationships between drug-resistance and transmission.¹²⁸ Furthermore, even where there are clear biological costs associated with resistance, these may be ameliorated by compensatory mutations.^{129, 130} WGS analyses of clinical strains suggest successful transmission of MDR strains in disparate settings such as South Africa¹³¹ and Russia¹³². However, a recent household contact study suggested circulating MDR-TB strains in Peru were less likely to result in disease in household contacts than drug sensitive strains.¹³³ The data on XDR transmission, at least during the time of observation, are also mixed.^{131, 134} Were the fitness of drug resistant strains to increase over time, these mixed results might reflect differences in the maturity of drug resistant TB epidemics.¹³⁵ Given the importance of reproductive potential to projections of the MDR and XDR-TB epidemics, this remains a research priority.

Antiretroviral therapy and MTB transmission: Our understanding of the effects of HIV on MTB transmission remains limited.⁷³ Some data suggest that HIV positive people with TB disease make a small contribution to MTB transmission. The arrival of HIV in Tanzania was associated with an increase in TB incidence but a decline in annual risk of TB infection measured in a series of tuberculin surveys.⁶⁷ The arrival of HIV in South Africa was associated with a marked increase in TB incidence in HIV positive but not HIV negative miners.¹³⁶ This finding was replicated in a prospective cohort study in business employees in Harare.¹³⁷ These studies were conducted before antiretroviral therapy was widely available. Household contact studies suggest transmission to household contacts is lower when the index case has more advanced HIV-related immunosuppression.¹³⁸ Molecular epidemiology suggests that, in a South African township, HIV negative people are more likely to be the index cases in strain clusters than HIV positive people.^{17, 139} However, a study in Malawi using WGS found no association between HIV status or receipt of ART and the probability of being linked to secondary cases.⁷⁶ There are several potential explanations for these observations. HIV positive people are more likely to have smear-negative or extrapulmonary disease, which are less infectious. Shorter disease duration¹⁴⁰ due to faster progression to death or treatment may limit opportunity to transmit, as might reduced social contact as a result of greater morbidity. However, whilst people in HIV care may have their TB diagnosed faster, healthcare facilities may be important sites of transmission.

Following publication of the START¹⁴¹ and ANRS TEMPRANO¹⁴² trials, the WHO guidelines have recently been updated.¹⁴³ It is now recommended that ART be provided to all people living with HIV irrespective of CD4 cell count. In response, national policies are likely to be updated to recommend earlier initiation of ART. ART reduces TB disease incidence rates in HIV cohorts by approximately two-thirds.¹⁴⁴⁻¹⁴⁶ Short-term reductions in population level TB disease rates have also been observed in communities in South Africa and Malawi where ART has been scaled up rapidly.^{147, 148} This may be largely explained by reduced progression from infection to disease rather than by reductions in transmission. The longer-term impact of ART on population-level TB disease burden and the impact on MTB infection incidence are uncertain. ART

certainly affects longevity, levels of contact with healthcare services and susceptibility to TB disease. It may also affect TB disease duration and phenotype, including the presence of cavities, smear positivity,^{149, 150} and the relative frequency of extra-pulmonary disease, all of which might affect infectiousness. Furthermore, reduced morbidity as a result of ART might result in increased levels of social contact.

An influential modelling study estimating the impact of the roll-out of annual HIV testing and immediate ART on TB disease incidence in nine African countries predicted a 21% (range: 10%–31%) reduction in the cumulative AIDS-related TB disease incidence over the first five years, and a 48% (range: 37%–55%) reduction in the incidence of TB disease at five years.¹⁵¹ A multi-model analysis for the time period 2014–2033, estimated that increasing ART coverage to 80% of those with CD4 count < 350 cells/mm³ could reduce TB incidence by 8–14% and, if ART were provided to all HIV infected individuals (at current levels of access), incidence could be reduced by 6–30%.¹⁵² However, a more recent modelling study suggested that the longer-term impact of expanded ART access was less certain.¹⁵³ Whilst TB incidence should initially decline, the model predicted that, if good adherence and immunologic responses to ART are not sustained and combined with effective HIV preventive interventions, increases in TB disease incidence might occur despite high levels of ART coverage.¹⁵³ Following publication of the new WHO guidelines,¹⁴³ it is likely that more people will be initiated on ART at higher CD4 counts. Given the substantial impact – either positive or negative – that this may have on TB in HIV endemic settings, the impact of HIV and ART on transmission dynamics should be a focus of research.

Conclusions

Addressing MTB transmission is critical to achieving TB control in high burden settings. Repeated surveys measuring TB infection in the same community, including young adults, offer a feasible measure of TB transmission. This approach might be used in trials in high burden settings to enable the impact of interventions on transmission to be disaggregated from their effects on rates of progression to disease or disease duration. The coming years will likely see innovative and exciting research on MTB transmission in high burden settings. When designing studies, researchers should be mindful of the context in such settings. Priority should be given to the development and evaluation of TB control strategies that place minimal additional demands on poor patients and overstretched healthcare systems,¹⁰⁹ and or that involve elements of social protection or health system strengthening.

SEARCH STRATEGY

To find articles pertinent to this narrative review, we searched PubMed to 1 October 2015. We sought articles published in English using the terms 'tuberculosis' and 'transmission'. We also included papers included in reference lists or suggested by members of the review team.

AUTHOR CONTRIBUTIONS

TAY, PYK, GMK, JGT, RGW, TC, FGC, DAJM and IA drafted sections of the manuscript. All authors commented on and edited the manuscript. TAY, PYK and IA prepared the final draft. All authors approved the final version of the manuscript prior to submission.

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Figure 1. The MTB transmission cycle.

Panel: Diagnostic tests of MTB infection

- There is no gold standard diagnostic test for MTB infection.
- The two widely used diagnostic tests are tuberculin skin tests (TSTs) and interferon-gamma release assays (IGRAs). Positive tests are interpreted as reflecting a prior adaptive immune response to mycobacterial infection.
- Currently it is not possible to detect infections cleared by the innate immune system prior to an adaptive response,⁴¹ nor to distinguish cleared infections that leave a lingering immunological footprint from persistent infection.
- Neither test is able to distinguish between latent infection and disease

Tuberculin skin test (TST)

- TSTs involve an intradermal injection of a standardised purified protein derivative (PPD) then measurement of any induration 48-96 hours later.
- Sensitivity and specificity are dependent on the number of millimetres of induration chosen as the cut point and the prevalence of non-specific reactions, which is determined by exposure to environmental mycobacteria and prior BCG vaccination.⁵⁷ The BCG effect wanes in children vaccinated in infancy.
- Low sensitivity can be seen with advanced age and with immunosuppression as a result of malnutrition or HIV.
- New statistical techniques can suggest appropriate cut points for given distributions of reactions sizes.⁵⁸
- Test reversions do occur and are commoner in young individuals, probably reflecting an initial false positive test.⁵⁹

Interferon gamma release assay (IGRA)

- IGRAs involve a blood draw. T cells are then exposed to antigens that are found in MTB but not in BCG nor in most environmental mycobacteria.
- Interferon-gamma released by cells that recognise these antigens is then assayed in the supernatant following incubation (Quantiferon Gold) or by counting the number of interferon gamma producing cells in an ELISPOT assay (T Spot TB).
- IGRAs are a more specific test for MTB infection but there is less precedent for their use in transmission studies. The need for phlebotomy and the high cost of the test are also disadvantages.
- Test reversions are common and the clustering of results around the threshold for positivity means the choice of cut point can substantially affect sensitivity, specificity and prevalence estimates.¹⁵

New tools

- A new and, hopefully, more specific skin test based on similar antigens to those used in IGRAs is now being tested in Phase III trials.⁶⁰
- RNA expression signatures have been developed that may distinguish disease from latent infection, TB from other diseases and that might revert following successful treatment of active TB.⁶¹⁻⁶³ These signatures require further validation.

Table 1. Measures of MTB transmission in populations.

Tool	What is measured	Advantages	Disadvantages
Prevalence of TB infection	Typically measured using TSTs in school going children	<p>Cheap and well established</p> <p>Infections must have occurred within an individual's lifetime, hence, in young children, this is a measure of recent infection</p> <p>Prevalence can be converted into an annualised incidence ('annual risk of tuberculosis infection', ARTI)⁵⁷</p> <p>Repeated surveys or continuous measurement of infection prevalence in the same age group can quantify changes in MTB transmission over time</p>	<p>Does not capture early clearance</p> <p>Poor sensitivity and specificity, uncertainty regarding cut points plus conversions and reversions can affect estimates in some populations</p> <p>From a single study, it is not possible to separate age and cohort effects</p> <p>'Styblo's rule', which states that there is a fixed relationship between ARTI and the incidence of TB disease, is no longer thought to be valid.^{64, 65}</p> <p>WHO no longer recommend single tuberculin surveys.</p>
Incidence of TB infection	Testing cohorts for TB infection longitudinally	<p>Older children can be included and inferences still made about recent transmission</p> <p>An incidence cohort including older children and adults provides more general insights into transmission in the community even if mixing patterns are strongly age assortative</p>	<p>Does not capture early clearance</p> <p>Requires larger sample or longer duration of follow up than measuring infection prevalence</p> <p>Losses to follow up may reduce power and bias estimates</p> <p>Excluding those who are positive at baseline may exclude those at highest risk – a particular problem in older individuals in high burden settings</p>
TB notifications	Notifications of TB disease to the national treatment programme	<p>Data are routinely captured</p> <p>It may be feasible to enhance capacity to diagnose and notify cases of TB for the purposes of research though substantial biases and quality problems inherent to routinely collected data are likely to persist</p>	<p>Serious problems with data quality in most high burden settings</p> <p>Only captures TB transmission that progresses to disease</p> <p>Only captures individuals who access a diagnosis and whose diagnosis is notified</p> <p>May capture individuals who do not have TB – poor specificity is a particular problem where TB diagnosis is primarily based on chest X-ray such as in children</p>
Prevalence of TB disease	Typically measured in large surveys using sputum culture with or without pre-screening for symptoms and or with a chest x-ray	<p>Well established</p> <p>Undiagnosed individuals can be referred for treatment</p>	<p>Substantial and expensive undertaking</p> <p>Only captures TB transmission that progresses to (pulmonary) disease</p> <p>It may not be clear whether changes in prevalence are a result of differences in transmission, in progression from infection to disease, or in disease duration</p> <p>Prevalence surveys are active case finding interventions and will transiently alter local TB epidemiology</p> <p>Sputum culture has low sensitivity in children</p>
Incidence of TB disease	Measured in established cohorts or using two prevalence surveys	<p>Allows changes in incidence to be disaggregated from changes in disease duration</p>	<p>Except in established cohorts in high burden settings, the measurement of incidence requires more than one large prevalence survey – this is rarely feasible</p> <p>Only captures TB transmission that progresses to (pulmonary) disease</p> <p>It may not be clear whether differences in prevalence are a result of differences in transmission or in progression from infection to disease</p>
Molecular epidemiology (proportion clustered)	The proportion of isolates that have the same strain type usually using RFLP, MIRU-VNTR or WGS *	<p>Allows inferences to be made about the proportion of TB resulting from reactivation vs. recent infection</p> <p>Strain typing can disprove or provide evidence to support putative transmission events</p>	<p>Requires advanced laboratory capacity</p> <p>Only captures transmissions that progress to disease and isolates that are sampled</p> <p>Biased estimates can be obtained if the sampling fraction is low, if the study is not of sufficient duration or if there is substantial in or out migration</p>

* RFLP is restriction fragment length polymorphism. MIRU-VNTR is Mycobacterial Interspersed Repetitive Units – Variable Number of Tandem Repeats. WGS is whole genome sequencing. These are all strain typing techniques.