



The transmission of *Mycobacterium tuberculosis* in high burden settings

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Unacceptable levels of *Mycobacterium tuberculosis* transmission are noted in high burden settings and a renewed focus on reducing person-to-person transmission in these communities is needed. We review recent developments in the understanding of airborne transmission. We outline approaches to measure transmission in populations and trials and describe the Wells–Riley equation, which is used to estimate transmission risk in indoor spaces. Present research priorities include the identification of effective strategies for tuberculosis infection control, improved understanding of where transmission occurs and the transmissibility of drug-resistant strains, and estimates of the effect of HIV and antiretroviral therapy on transmission dynamics. When research is planned and interventions are designed to interrupt transmission, resource constraints that are common in high burden settings—including shortages of health-care workers—must be considered.

Introduction

Sustained reductions in disease incidence of up to 20% per year are required to meet the targets set out in the WHO End TB Strategy.^{1,2} However, incidence is currently only estimated to be reducing at 1·5% per annum.³ This trend is consistent with model predictions with respect to the probable effect of present 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 is an issue. Although quality data for active tuberculosis in children younger than 5 years are scarce, the incidence of paediatric cases indicate continuing high levels of transmission.^{3,6,7} Tuberculin surveys in high prevalence countries estimate annual risks of *Mycobacterium tuberculosis* infection of 0·3–2·2%,^{8–12} but exceeding 5% 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 might underestimate transmission.¹⁵ Data for *M tuberculosis* transmission derived from 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 tuberculosis,^{16,17} particularly in people living with HIV.¹⁸ The rapid rebound in tuberculosis incidence after the discontinuation of isoniazid preventive treatment (IPT) in southern African studies suggest continuing transmission is important in high burden settings,^{19,20} although models predict a contribution from reactivation disease implying IPT might not sterilise.^{21,22}

To achieve the goals of the End TB Strategy,² an increased emphasis on reducing person-to-person *M tuberculosis* transmission in high burden settings is needed. This Review summarises research into *M tuberculosis* transmission in these settings. We focus on the biology of airborne *M tuberculosis* transmission, measuring transmission in populations, and modelling transmission with the Wells–Riley approach. We conclude

by identifying research priorities. We do not discuss transmission-blocking vaccines or mixed infections, each the subject of a recent review article.^{23,24} Of note, no international consensus exists for tuberculosis incidence or prevalence thresholds that define high burden, although a tuberculosis incidence of 100 cases per 100 000 people per year has been used by WHO.²⁵ Most of the studies we review were implemented in communities with a tuberculosis incidence of 100 cases or more per 100 000 people per year.

Airborne *M tuberculosis* transmission

Although *M tuberculosis* complex organisms can be spread through unpasteurised milk, direct inoculation, and other means, we focus on the predominant route, airborne transmission. The fundamentals of airborne *M tuberculosis* transmission were described by William Frith Wells, Richard Riley, Robert Loudon, Rena Roberts, and others, more than 60 years ago.²⁶ Recent progress in basic and clinical sciences has improved our understanding of *M tuberculosis* transmission, which had remained largely unchanged for more than 50 years. However, much remains unknown. Interruption of any process in the natural history of *M tuberculosis* will reduce rates of transmission at a population level (figure).

Individuals with pulmonary tuberculosis aerosolise *M tuberculosis*, placing their contacts at risk of infection (figure). This aerosolisation occurs at a faster rate during coughing.²⁷ Some evidence suggests that speech and singing are effective aerosol generating activities,^{28,29} although these studies focused only on droplets originating in the mouth. Although the largest respiratory droplets fall to the ground, rapid evaporation means many droplets attain a sufficiently low mass before settling so that they remain suspended in air currents until either inhaled or ventilated out of the room.³⁰ Important new insights into *M tuberculosis* transmission have come from cough box experiments.³¹ In these studies, tuberculosis patients were asked to cough as frequently as was comfortable for 5 min

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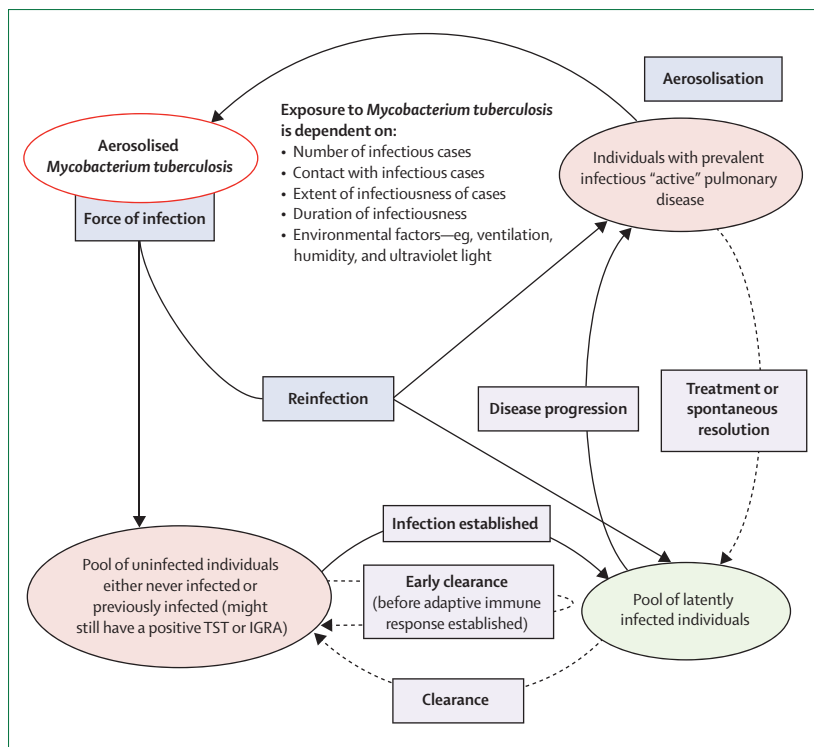


Figure: *Mycobacterium tuberculosis* transmission cycle
IGRA=interferon-gamma release assay. TST=tuberculin skin test.

positive tuberculin skin tests (TSTs) or interferon-gamma release assays (IGRAs) and the discovery of genetic loci that predict TST positivity suggest that the body can clear *M tuberculosis* infection without development of an adaptive immune response—so-called early clearance.⁴² This process is probably important epidemiologically but, because no evidence of the early clearance is left, is difficult to study. Informed by animal studies and advanced imaging techniques, appreciation is growing for the opinion that a binary classification of tuberculosis into latent infection and active disease might be too simplistic.^{43,44} Some individuals with positive tests for infection might have cleared the mycobacteria and periods of active replication in latent tuberculosis have been reported.^{43,44} Pulmonary *M tuberculosis* infection towards the more active end of the infection-to-disease range is probably necessary for infectiousness.

A widely-held view is that the infectiousness of patients diminishes sufficiently after 2 weeks of antituberculous treatment such that transmission to contacts is unlikely.⁴⁵ Many guidelines rely on proxy measures of infectiousness—eg, smear status or culture conversion. Of note, median time to culture conversion in patients treated with daily direct observation for drug-susceptible tuberculosis in Peru was much longer than 2 weeks at 37 days.⁴⁶ However, in patients on treatment, the association between sputum smear or culture status and infectiousness is not straightforward,^{47–51} and is likely to be influenced not only by mycobacteria viability in respiratory secretions but also by the capacity to generate aerosolised *M tuberculosis* through coughing.²⁷ Because cough frequency diminishes with treatment,⁵² assumptions about infectiousness on the basis of culture conversion times might overestimate risk. Furthermore, organisms that propagate in culture might not thrive when exposed to a hostile immune system in the alveoli.

Infectiousness can be studied in guineapig facilities in which the number of animals infected after exposure to air exhausted from isolation rooms containing patients with tuberculosis is measured. Such experiments show that effective treatment is associated with decidedly fewer *M tuberculosis* infections than are reported before treatment is initiated or when isolates are not fully susceptible to the treatment regimen.^{34,51} However, the guineapigs in these experiments were exposed to the patients for many weeks. Thus, these experiments have not yet reliably established a time window after which a patient can be deemed to be no longer infectious. Most, if not all, household contacts are more likely to be infected by an index patient in the pretreatment period than once treatment is initiated due to both the likely longer duration of exposure and the greater infectiousness pretreatment.⁵³ This situation might also be true for patients with drug-resistant tuberculosis, in whom culture conversion times will typically be longer than those with drug-susceptible tuberculosis, particularly if initiation of effective treatment is delayed.

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into a cough aerosol sampling system. Although this procedure might not represent real physiological processes, these experiments suggest that most *M tuberculosis* 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 guineapig facilities^{33,34} and molecular epidemiological observations,^{35–37} suggest that some people with tuberculosis might be much more infectious than others. Early animal experiments showed that *M tuberculosis* in smaller droplets more readily produced tubercles in the lung than did *M tuberculosis* in larger droplets, presumably because these small droplets escape filtration in the upper airways.^{38,39} The use of surgical masks by patients with tuberculosis has been shown to reduce transmission to guineapigs by 56%, suggesting they partly block aerosolisation of the relevant respiratory droplets.⁴⁰

The quantity and characteristics of the inhaled droplet can predict clinical outcomes, with early experiments showing that the infectious dose predicts the risk of infection and progression to disease.⁴¹ In the cough box experiments, the quantity of aerosolised *M tuberculosis* produced by individuals predicted infection in household contacts better than the smear grade or time to culture positivity.³² 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.⁴¹ The fact that some highly exposed individuals do not develop

Measuring transmission in populations

Even in the highest burden communities, the prevalence and annual incidence of active tuberculosis disease rarely exceed 2%. Infrequent outcomes, in combination with incomplete surveillance data and poor tests for infection, make the measurement of *M tuberculosis* transmission in populations a challenge.⁵⁴ Inference about transmission therefore relies on proxy measures, assumptions, and combination approaches. The best approach to measure *M tuberculosis* transmission in trials of control interventions is controversial.^{55–57} Short-term reductions in disease prevalence, for example, are difficult to interpret as prevalence is affected not only by transmission but also by progression from infection to disease and disease duration.⁵⁵ We have summarised frequently used tests for *M tuberculosis* infection (panel) and approaches to measure the *M tuberculosis* transmission in populations (table).

Traditional approaches to measure transmission include tuberculin surveys in schoolchildren. Such surveys are a typical means of estimating *M tuberculosis* transmission at a population level. Although age assortative mixing might mean paediatric infections do not fully reflect *M tuberculosis* transmission between adults,^{55,58,68} 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 vaccine scars or the cut point used to define a positive test for infection.^{67,69}

Molecular approaches include methods for strain typing *M tuberculosis* such as spoligotyping, which has low resolution; restriction fragment length polymorphism and mycobacterial interspersed repetitive units-variable number of tandem repeats,^{70–72} both of which have been widely used; and whole genome sequencing (WGS). These molecular epidemiological techniques provide evidence for or against potential linkages between two or more cases of active tuberculosis and have led to several crucial insights into *M tuberculosis* transmission.⁷³ Since these techniques need a bacterial isolate, molecular epidemiology, with rare exceptions,⁷⁴ captures only infections that have progressed to active tuberculosis disease.⁷⁵ Molecular epidemiology cannot distinguish changes in transmission intensity from changes in the rate of progression to active tuberculosis shortly after infection resulting, for example, from varying levels of immunosuppression. The high resolution of WGS and steep reductions in cost mean this technique is likely to eventually replace existing strain typing techniques. However, molecular epidemiology with WGS will need an understanding of the rate at which mutations occur. Recent studies^{37,76–78} suggest that, in active disease, single nucleotide polymorphisms (SNPs) emerge, on average, at half an SNP per *M tuberculosis* genome per year or slower. Most of the patients in these studies were on tuberculosis treatment and substantial variation was reported in the

Panel: Diagnostic tests of *Mycobacterium tuberculosis* infection

- No gold standard diagnostic test exists for *Mycobacterium tuberculosis* infection.
- The two widely used diagnostic tests are tuberculin skin tests (TSTs) and interferon-gamma release assays (IGRAs). Positive tests are interpreted as showing a previous adaptive immune response to mycobacterial infection.
- Detection is not possible for infections cleared by the innate immune system before an adaptive response,⁴² nor is it possible 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 tests

- TSTs use an intradermal injection of a standardised purified protein derivative then measurement of any induration after 48–96 h.
- Sensitivity and specificity are dependent on the number of millimetres of induration chosen as the cut point and the prevalence of non-specific reactions resulting from exposure to environmental mycobacteria or previous BCG vaccination.⁵⁸ 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 reaction sizes.⁵⁹
- Test reversions do occur and are more common in young individuals, probably reflecting an initial false positive test.⁶⁰

Interferon-gamma release assay

- IGRAs require a blood sample to be taken from patients. T cells are then exposed to antigens that are found in *M tuberculosis* but not in BCG or in most environmental mycobacteria.
- Interferon-gamma released by cells that recognise these antigens is then assayed in the supernatant after incubation or by counting the number of interferon-gamma producing cells in an enzyme-linked immunospot assay.
- IGRAs are a more specific test for *M tuberculosis* infection but less precedent exists 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 methods

- A new and, hopefully, more specific skin test based on similar antigens to those used in IGRAs is now being tested in phase 3 trials.⁶¹
- RNA expression signatures have been developed that might distinguish disease from latent infection, tuberculosis from other diseases, and that might revert after successful treatment of active tuberculosis.^{62–65} These signatures need further validation.

rate at which mutations occurred. A primate study suggests a similar mutation rate and that the mutation rate might not differ substantially between active and latent infection.⁷⁹ However, scarce data suggest that, in man, mutations accumulate more slowly during latent infection than in active infection.⁸⁰ Occasional accelerated inpatient microevolution events⁸¹ and the slow rate at which SNPs accumulate might make inference of chains of transmission from *M tuberculosis* genotypes alone challenging. Probabilistic models that also incorporate epidemiological and clinical data might be needed.⁸² With molecular epidemiology by WGS, as with older strain

	What is measured?	Advantages	Disadvantages
Prevalence of tuberculosis infection	Typically measured with tuberculin skin tests in school-age children	Cheap and well established; infections must have occurred within an individual's lifetime, hence, in young children, this test is a measure of recent infection; prevalence can be converted into an annualised incidence (ie, ARTI); ⁵⁸ repeated surveys or continuous measurement of infection prevalence in the same age group can quantify changes in <i>Mycobacterium tuberculosis</i> transmission over time	Does not capture early clearance; poor sensitivity and specificity, uncertainty with respect to cut points plus conversions and reversions of test positivity can affect estimates in some populations; from a study at only one timepoint, age and cohort effects cannot be separated; Styblo's rule, which states that ARTI and the incidence of tuberculosis disease have a fixed association, is no longer thought to be valid; ^{66,67} WHO no longer recommends single tuberculin skin test surveys
Incidence of tuberculosis infection	Testing cohorts for tuberculosis infection longitudinally	Older children can be included and inferences still made about recent transmission; an incidence cohort including older children and adults provides a general insight into transmission in the community even if mixing patterns are strongly age assortative	Does not capture early clearance; needs larger sample or longer duration of follow-up than measurements of infection prevalence; losses to follow-up might reduce power and bias estimates; exclusion of those who are positive at baseline might exclude those at highest risk—a particular issue in older individuals in high burden settings
Tuberculosis notifications	Notifications of tuberculosis disease to the national treatment programme	Data are routinely captured; enhancement of capacity to diagnose and notify cases of tuberculosis might be possible for the purposes of research, although substantial biases and quality problems inherent to routinely collected data are likely to persist	Serious problems with data quality in most high burden settings; captures only tuberculosis transmission that progresses to disease; captures only individuals who access a diagnosis and whose diagnosis is notified; might capture individuals who do not have tuberculosis—poor specificity is a particular issue where tuberculosis diagnosis is mainly on the basis of chest radiographs, such as in children
Prevalence of tuberculosis disease	Typically measured in large surveys by sputum culture with or without prescreening for symptoms and/or with a chest radiograph	Well established; undiagnosed individuals can be referred for treatment	Substantial and expensive undertaking; captures only tuberculosis transmission that progresses to (pulmonary) disease; whether changes in prevalence are a result of differences in transmission, in progression from infection to disease, or in disease duration, might not be clear; prevalence surveys are active case finding interventions and will transiently change local tuberculosis epidemiology; sputum culture has low sensitivity in children
Incidence of tuberculosis disease	Measured in established cohorts or by two prevalence surveys	Allows changes in incidence to be disaggregated from changes in disease duration	Except in established cohorts in high burden settings, the measurement of incidence needs more than one large prevalence survey, which is rarely feasible; captures only tuberculosis transmission that progresses to (pulmonary) disease; whether differences in incidence are a result of variation in transmission or in progression from infection to disease is not clear
Molecular epidemiology (proportion clustered)	The proportion of isolates that have the same strain type usually with RFLP, MIRU-VNTR, or WGS*	Allows inferences to be made about the proportion of tuberculosis resulting from reactivation versus recent infection; strain typing can disprove or provide evidence to support putative transmission events	Needs advanced laboratory capacity; captures only transmissions that progress to disease and isolates that are sampled; biased estimates can be obtained if the sampling fraction is low, if the study is not of sufficient duration, or if substantial in or out migration of participants takes place

ARTI=annual risk of tuberculosis infection. RFLP=restriction fragment length polymorphism. MIRU-VNTR=mycobacterial interspersed repetitive units-variable number of tandem repeats. WGS=whole genome sequencing. *These techniques type strains of *M tuberculosis*.

Table: Measures of *Mycobacterium tuberculosis* transmission in populations

typing techniques, adequate study duration, a high sampling fraction, and careful documentation, follow-up, and reporting are important.^{16,83–85} However, novel approaches to the analysis of sequence data might allow population level inferences to be made from a smaller number of people than the older genotyping techniques.

Direct detection of aerosolised *M tuberculosis* in samples of room air is of great interest. This ability might allow quantification of *M tuberculosis* exposure in putative sites of transmission. A few demonstration studies^{86–88} of PCR with room air filtrate suggest that this detection might be feasible. Although PCR detection of *M tuberculosis* DNA does not necessarily mean organisms are viable, it would suggest that individuals with pulmonary tuberculosis have produced bioaerosols in the space. This finding should be, at least in theory, a reasonable proxy for transmission risk.

The Wells–Riley equation

Room ventilation and social contact patterns predict whether other individuals are exposed to *M tuberculosis* that has been aerosolised. The Wells–Riley equation is used to model the transmission of respiratory pathogens,⁸⁹ such as *M tuberculosis*, that are spread by crowd rather than close contact. Transmission risk in a defined space over time t is modelled as a Poisson process:

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

where I is the number of infectious individuals present, q is the rate at which infectious individuals produce 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”.⁸⁹ The parameter q is often assumed or fitted to data. Various attempts have been made to empirically estimate q for tuberculosis by venting air exhaled by patients with tuberculosis over experimental animals. Two sets of experiments in the pre-HIV era estimated quanta production at 0.62–0.82 and 1.25 per h with a heterogeneous group of tuberculosis patients.^{40,90–92} More recently, Escombe and colleagues³⁴ obtained an estimate of 8.2 quanta per h in a group of patients living with HIV in Lima, Peru. These averages disguise huge heterogeneity in infectiousness with the most infectious patients in each study producing 60 and 226 infectious quanta per h.^{34,91,93} High rates of quanta production have been measured in patients with advanced multidrug-resistant tuberculosis,⁴⁰ and very high rates estimated in outbreaks related to aerosol generating procedures.⁹⁴ Interestingly, estimates of q obtained by fitting to data from high burden communities are lower than those obtained empirically.⁹⁵ This finding might 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 several important limitations. The equation 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 a rebreathed fraction, the fraction of inhaled air that has been exhaled previously by someone in the building.⁹⁶ This rebreathed fraction can be obtained from paired indoor and outdoor carbon dioxide (CO_2) measurements. This process avoids the need to measure Q , which can be technically challenging. In many settings, spatial and temporal variations in CO_2 concentrations are substantial. Not obtaining contemporaneous CO_2 measurements from directly outside the buildings studied might be reasonable in the absence of alternative CO_2 sources and where wind speeds restrict local spatial and temporal variations in CO_2 .^{95,97} The absence of such contemporaneous measurements would not be reasonable in other circumstances. Several important insights have been derived from the Wells–Riley approach. For example, one study⁹⁸ suggested that active case finding could not control high levels of *M tuberculosis* transmission in a South African prison if levels of overcrowding and poor ventilation were not also addressed. Another South African study,⁹⁵ which used the equation to predict settings in which *M tuberculosis* transmission might occur, is described later in this Review.

Research priorities

Much is still to be learned about *M tuberculosis* transmission. Approaches to interrupting *M tuberculosis*

transmission include active case finding, the provision of IPT, and tuberculosis infection control. Large trials have been completed into active case findings and mass IPT to interrupt *M tuberculosis* transmission. The ZAMSTAR⁵⁶ result may be the first empirical data suggesting that active screening for tuberculosis disease reduces *M tuberculosis* transmission at a population level.⁹⁹ The Thibela tuberculosis trial,²⁰ implemented in a setting with a very high incidence of infection, reported mass administration of IPT protected individuals whilst on treatment but had no effect on tuberculosis incidence in the wider community.²²

Infection control

Little research into tuberculosis infection control has been undertaken. Tuberculosis infection control is conventionally described in three domains—administrative controls (which aim to minimise contamination of shared air by infectious subjects—eg, cough triage, early diagnosis, and treatment), environmental controls (which aim to minimise exposure to *M tuberculosis* through disinfection or removal of contaminated air), and personal protection measures (which aim to minimise inhalation of contaminated air—eg, N95 respirators).¹⁰⁰ A review of observational and animal studies¹⁰¹ concluded that the evidence is strong in support of the role of ventilation as an environmental control in reducing the risk of airborne transmission of *M tuberculosis*. Of the many ways to increase ventilation, increased mechanical ventilation,¹⁰² natural ventilation through increased window opening,¹⁰³ and wind-driven roof turbines have been considered specifically as means to reduce *M tuberculosis* infection risk.¹⁰⁴ Natural ventilation has been recommended by WHO as an effective way to reduce infection.¹⁰⁰ Air disinfection methods, particularly upper room ultraviolet germicidal irradiation, have also been studied leading to steep reductions in *M tuberculosis* transmission from tuberculosis patients to experimental animals.^{105,106}

Implementation of 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 high running and maintenance costs of mechanical systems, are side-effects of increased ventilation¹⁰⁷ and might make such measures unacceptable to occupants. Therefore, the ideal retrofit and design measures used 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 instruments might be used to predict the optimum design or retrofit of buildings to maximise ventilation according to specified criteria.^{108,109}

The FAST approach to tuberculosis infection control in congregate settings has been promoted and advocates

“Finding TB cases Actively, Separating safely, and Treating effectively”.¹¹⁰ A trial of the FAST approach is about to commence in Peru (NCT 02355223) using TST negative to positive conversions in health-care workers as an endpoint. However, the absence of a comparator group might limit the strength of the conclusions that can be reached. Although an association between ventilation rate and tuberculosis transmission is clear, little empirical evidence exists for the effectiveness of infection control interventions in reducing transmission, with most studies using animal surrogates or ventilation measurements as a proxy for transmission risk. A notable exception was the Tuberculosis Ultraviolet Shelter Study,¹¹¹ which showed that environmental modifications can be safely implemented at scale.¹¹¹ Although too few TST conversions occurred in residents of the shelters to show an effect on *M tuberculosis* transmission, similar studies in high burden settings would be valuable to quantify the effect of tuberculosis infection control interventions on transmission to human occupants and, potentially, on transmission in the surrounding community.¹¹² Whether household infection control implemented at diagnosis can mitigate against secondary infections in patients managed in the community is not known. To our knowledge, no trials of such interventions have been implemented. This question is important, perhaps in multidrug-resistant tuberculosis and certainly in extensively drug-resistant tuberculosis, in which chemotherapy might not promptly reduce infectiousness and where the consequences of transmission might be severe.

Locating *M tuberculosis* transmission

Historically, households have been deemed to be a major focus of *M tuberculosis* transmission. However, three molecular epidemiological studies^{113,114} from sub-Saharan Africa and one from rural Vietnam,¹¹⁵ all suggest that most transmission occurs between, rather than within, households. In these high burden settings, this finding probably 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 transmissions occur outside the household,^{116,117} but studies suggest this result is age dependent with young children more likely to have been infected by a household member.^{118–120} Transmission in indoor congregate settings is probably important in high burden settings. For example, time working in public transport is strongly associated with TST positivity in Lima, Peru.¹²¹ Understanding which settings are important should be a research priority, because this knowledge would allow infection control and active case finding interventions to be better targeted.¹¹² The Wells–Riley equation and its variants have been used to estimate *M tuberculosis* transmission risk by location. Studies adopting these approaches have estimated ventilation or likely exposure to exhaled bioaerosols

based on CO₂ levels. These methods have been applied to study transmission in a Cape Town, South Africa, township. Data were collected for CO₂ concentrations in various settings visited by 63 adolescents. 93% of total exposure to rebreathed air was estimated to occur in a few locations: own home, visited homes, public transport, work, or school.⁹⁷ The same research group used a modified Wells–Riley equation, CO₂ measurements, and social contact pattern data to estimate the proportion of overall *M tuberculosis* transmission by location. The researchers reported that, in the same Cape Town township, 50% of incident infections in 15–19 year olds might take place in school, that workplaces are important places for adult transmission, and that household and public transport might be important sites of transmission between age groups.⁹⁵ These inferences are potentially useful but the studies were small in terms of geographical extent and participant numbers. The conclusions might be context specific and assume tuberculosis 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 tuberculosis prevalence.

Health facilities, particularly in HIV endemic areas, are an important setting in which patients infectious with tuberculosis mix with susceptible people. People with infectious tuberculosis attend health-care facilities before diagnosis, when presenting with tuberculosis symptoms, and during the course of tuberculosis 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 people at risk of progressing from infection to disease in settings where the likelihood of exposure to patients with infectious tuberculosis is relatively high. In a study¹²² based in an emergency department in Lima, Peru, IGRA conversion was reported in 30% of health-care workers during a 12 month period compared with 0% of hospital security and domestic personnel; remarkably a third of patients identified with tuberculosis in the study were attending the hospital for an apparently unrelated reason (trauma, pregnancy, etc) showing the importance of the unrecognised risk. Nosocomial transmission played an important part in the Tugela Ferry, South Africa, extensively drug-resistant tuberculosis outbreak.¹²³ Future research should quantify the proportion of *M tuberculosis* transmission in high burden settings that occurs within health-care facilities and the effect of programmatic infection control interventions on this proportion.

Spatial heterogeneity in the incidence of tuberculosis and drug-resistant tuberculosis is evident in analyses of programmatically collected data and has been well documented in many studies,^{124–126} raising the possibility that targeted interventions might be effective. At this time, however, the mechanisms driving such heterogeneity are

not completely understood—eg, localised transmission and/or aggregation of individuals sharing risk factors for infection or progression might combine to generate such patterns of disease. An improved understanding of this spatial heterogeneity might usefully inform targeted tuberculosis control interventions. Improved data for the geographical extent of social contacts relevant for tuberculosis transmission would assist in the design of intervention studies. Research addressing this question would be valuable.

Drug resistance and transmissibility

Globally, an estimated 480 000 incident cases of multidrug-resistant tuberculosis occurred in 2013. The proportion of new cases that are infected with multidrug-resistant tuberculosis is about 3·5% and this percentage has not changed noticeably over the period of 2008 to 2013.³ Multidrug-resistant tuberculosis is disproportionately distributed, with the highest rates reported in central Asia and eastern Europe where, in several countries, a high proportion of multidrug-resistant tuberculosis cases have no previous history of tuberculosis treatment.³ This finding suggests high levels of multidrug-resistant tuberculosis transmission.

Projections of the future burden of multidrug-resistant tuberculosis depend crucially on estimates of the reproductive potential of drug-resistant strains as compared with drug-sensitive strains.^{127,128} 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.¹²⁹ Drug resistance might affect several of these factors. For example, the duration of infectiousness is often longer in individuals with multidrug-resistant tuberculosis because delays in the diagnosis of resistance can lead to delayed initiation of effective treatment. However, the probability of exposure causing a secondary infectious case might be decreased if resistance-conferring mutations have a fitness cost.¹³⁰ In-vitro experiments (eg, competitive growth assays that measure biological fitness) and observational studies (eg, contact tracing and molecular clustering studies, which measure effects of biological fitness and differences in duration of infectiousness) suggest a wide variation in the association between drug resistance and transmission.¹³¹ Furthermore, even where clear biological costs are associated with resistance, these costs can be ameliorated by compensatory mutations.^{132,133} WGS analyses of clinical strains suggest successful transmission of multidrug-resistant strains in disparate settings, such as South Africa and Russia.^{134,135} However, a household contact study¹³⁶ suggested circulating multidrug-resistant tuberculosis strains in Peru were less likely to result in disease in household contacts than drug-sensitive strains. The data for transmission of extensively drug-

resistant *M tuberculosis*, at least during the time of observation, are also mixed.^{134,137} Because the fitness of drug resistant strains might increase over time (as a result of selection or through compensatory mutations), these mixed results might reflect differences in the maturity of drug-resistant tuberculosis epidemics.¹³⁸ In view of the importance of reproductive potential to projections of the multidrug-resistant and extensively drug-resistant tuberculosis epidemics, this topic is still a research priority.

HIV and *M tuberculosis* transmission

Our understanding of the effects of HIV on *M tuberculosis* transmission is scarce.⁷⁵ Some data suggest that people living with HIV with tuberculosis disease make a small overall contribution to *M tuberculosis* transmission. The arrival of HIV in Tanzania was associated with an increase in tuberculosis incidence but a reduction in annual risk of tuberculosis infection measured in a series of tuberculin surveys.⁶⁹ The arrival of HIV in South Africa was associated with an obvious increase in tuberculosis incidence in people living with HIV but not those who were HIV negative.¹³⁹ This finding was replicated in a prospective cohort study¹⁴⁰ in business employees in Harare. These studies were completed before antiretroviral therapy was widely available. A household contact study¹⁴¹ suggests that transmission to household contacts is less frequent when the index case has more advanced HIV-related immunosuppression. 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.^{17,142} However, a study⁷⁸ in Malawi with WGS reported no association between HIV status or receipt of antiretroviral therapy (ART) and the probability of being linked to secondary cases. Several potential explanations exist for these observations. People living with HIV are more likely to have smear-negative or extrapulmonary disease, which are less infectious. Short disease duration due to fast progression to death or treatment might limit the opportunity to transmit,¹⁴³ as might the reduced social contact as a result of greater morbidity. However, although people in HIV care might have their tuberculosis diagnosed faster, health-care facilities might be important sites of transmission.

After publication of the START¹⁴⁴ and ANRS TEMPRANO¹⁴⁵ trials, WHO guidelines have been updated.¹⁴⁶ ART is now recommended to be provided to all people living with HIV irrespective of CD4 cell count. In response, national policies are likely to be updated to recommend an earlier initiation of ART than recommended at present. ART reduces tuberculosis disease incidence rates in HIV cohorts by about two-thirds.^{147–149} Short-term reductions in population level tuberculosis disease rates have also been reported in communities in South Africa and Malawi, where ART has been scaled up rapidly.^{150,151} This finding might be largely explained by reduced progression from infection to disease rather than by reductions in transmission.

Search strategy

We searched all studies published before Oct 1, 2015, in PubMed. We sought articles published in English using the terms “tuberculosis” and “transmission”. We also included papers from the reference lists of these papers and all authors suggested papers for inclusion in the Review.

The long-term effect of ART on tuberculosis disease burden at the population level and the effect on *M tuberculosis* infection incidence are uncertain. ART certainly affects longevity, levels of contact with health-care services, and susceptibility to tuberculosis disease. ART might also affect tuberculosis disease duration and phenotype, including the presence of cavities, smear positivity,^{152,153} and the frequency of extrapulmonary 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 study¹⁵⁴ estimating the effect of the roll-out of annual HIV testing and immediate ART on tuberculosis disease incidence in nine African countries predicted a 21% (range 10–31%) reduction in the cumulative AIDS-related tuberculosis disease incidence over the first 5 years, and a 48% (range 37–55%) reduction in the incidence of tuberculosis disease at 5 years. A multimodel analysis¹⁵⁵ for the time period 2014–33, estimated that increasing ART coverage to 80% of those with a CD4 count of 350 cells per μL or less could reduce tuberculosis incidence by 8–14%. Additionally, if ART were provided to all HIV infected individuals (at present levels of access), incidence could be reduced by 6–30%. However, a more recent modelling study¹⁵⁶ suggested that the long-term effect of expanded ART access was less certain than the earlier models suggested. Although tuberculosis incidence should initially reduce, the model predicted that, if good adherence and immunological responses to ART are not sustained and combined with effective HIV preventive interventions, increases in tuberculosis disease incidence might occur despite high levels of ART coverage.¹⁵⁶ After publication of the new WHO guidelines,¹⁴⁶ more people will probably start treatment with ART at higher CD4 counts than previously. In view of the substantial effect—either positive or negative—that this new practice might have on tuberculosis in HIV endemic settings, the effect of HIV and ART on transmission dynamics should be a focus of research.

Conclusions

Addressing *M tuberculosis* transmission is crucial to achieve control of tuberculosis in high burden settings. Repeated surveys measuring tuberculosis infection in the same community, including young adults, offer a feasible measure of tuberculosis transmission. This approach might be used in trials in high burden settings to enable the effect of interventions on transmission to be

disaggregated from their effects on rates of progression to disease or disease duration. The coming years will see innovative and exciting research on *M tuberculosis* transmission in high burden settings. Priority should be given to the development and assessment of strategies for tuberculosis control that place minimal additional demands on poor patients and overstretched health-care systems,¹¹² or that include elements of social protection or health system strengthening.

Contributors

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 before submission.

Declaration of interests

We declare no competing interests.

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For more about the transmission meeting see <http://tb.lshtm.ac.uk/tb-transmission>

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