



# Tuberculosis

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**ABSTRACT** This article reviews the published literature on tuberculosis from September 2012 to August 2013 and describes important advances in tuberculosis epidemiology, microbiology, pathology, clinical pharmacology, genetics, treatment and prevention.



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This paper reviews the published literature from September 2012 to August 2013 and describes important advances in TB <http://ow.ly/siTLk>

## Introduction

A number of important contributions have been made to the literature on tuberculosis (TB) in the past year. This article reviews the literature and summarises the most important contributions in the following areas: epidemiology, microbiology, pathology, clinical pharmacology, genetics, treatment and prevention.

## Epidemiology

Several important observations have been made about TB transmission in the past year. In an innovative investigation from South Africa, WOOD *et al.* [1] studied social mixing patterns among persons living in a township with a high incidence of TB. 571 persons completed a detailed 24-h diary of the duration and number of persons with whom they spent time indoors, as well as the location of the encounter. Surprisingly, most contacts were generated by sharing air on public transport (27.1%), while time spent sharing air in households generated 25.1% of contacts and time spent in the workplace and community buildings generated only 8% and 6% of contacts, respectively. There was substantial variation by age, with school contacts predominating in persons aged <19 years. The low proportion of work contacts may reflect high unemployment in the township studied. These results indicate that social mixing is far from random, and that public transport may be a high-risk location for TB transmission.

A second study by this group further explored the relationship between public transport and TB transmission using a modified Wells–Riley equation to model the risk of transmission on minibus taxis, buses and trains [2]. Carbon dioxide concentrations experienced by riders were sampled using a portable continuous sampling device that sampled at 5-s intervals and the number of riders per trip was assessed. Carbon dioxide levels were 2.5 to 4.5 times higher in transport vehicles compared to outdoor air (mean concentration 1800 ppm in minibus taxis, 1150 ppm in buses and 1000 ppm in trains, compared with 410 ppm in outdoor air). Because nearly all rebreathed carbon dioxide comes from that exhaled by other riders, these levels were used as a surrogate for rebreathed air. Using the Wells–Riley equation, the risk of infection was estimated to be highest in minibus taxis; the model predicted that it could be as high as 3.5% to 5% per year. Thus, public transport must be seen as an important potential location for TB transmission.

A second topic on which an important contribution was made was the use of whole-genome sequencing of *Mycobacterium tuberculosis* in tracking TB transmission. In an elegant analysis of 86 isolates collected over a

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13-year period, ROETZER *et al.* [3] determined that traditional genotyping failed to discriminate two distinct outbreaks with closely related strains, whereas whole gene sequencing clearly showed two distinct chains of transmission. This result was more closely aligned with the results of contact investigations. Moreover, the investigators were able to track single nucleotide changes in *M. tuberculosis* isolates over time in successive human hosts. They conclude that the organism accumulates mutations at a rate of approximately 0.4 mutations per genome per year. Thus, whole-genome sequencing provided better discrimination and correlated more closely with contact tracing information than IS 6110 or mycobacterial interspersed repetitive unit variable number tandem repeat typing. With the increasing availability and decreasing cost of whole-genome sequencing technology, it appears likely that we will see more advances in our understanding of the molecular events associated with TB transmission in the coming years.

### Microbiology

CHIGUTSA *et al.* [4] used the number of days it took for individual patient sputum specimens to become positive in Mycobacteria Growth Indicator Tube (Beckton, Dickinson and Co., Sparks, MA, USA) as a surrogate for initial organism burden. They studied a prospective cohort of 144 patients being treated for TB to describe the time course of reduction of bacteria in sputum from patients [4]. The authors showed that the data were best modelled by a bi-exponential decline, confirming the results of studies using serial dilution on solid agar, a time-consuming technique that is difficult to standardise. These results imply that there are two populations of bacteria in the sputum of patients with pulmonary TB, and the authors estimate that the rapidly killed subpopulation predominates early but is rapidly eliminated, with a half-life on treatment of only 1.8 days. In contrast, the slowly killed subpopulation has a half-life on treatment of 39 days and, therefore, predominates after week 2. The significance of these observations are that such modelling, using readily available data, may help design and test TB drug regimens that could target both populations, thus increasing therapeutic efficiency.

### Pathology

It has long been believed that *M. tuberculosis* organisms persist in an inactive or latent state in specific loci in persons with a positive skin test but no evidence of disease; however, this fact has never been directly demonstrated. A study by BARRIOS-PAYÁN *et al.* [5] provides direct evidence of this phenomenon. The investigators studied lung specimens from 49 persons in Mexico who died of causes other than TB. Tissue was hybridised with *M. tuberculosis*-specific probes to identify sites that harboured inactive TB using *in situ* PCR, real-time PCR and spoligotyping. 43 (88%) out of 49 persons had evidence of inactive TB in at least one location; *M. tuberculosis* DNA was identified in 36 lung samples, 35 spleen samples, 34 kidney samples and 33 liver samples, but not all subjects were positive at all sites. Using *in situ* PCR, a variety of cell types were found to harbour *M. tuberculosis*, including the endothelium, pneumocytes and macrophages in the lung, Bowman's parietal cells and convoluted proximal tubules in the kidney, macrophages and sinusoidal endothelial cells in the spleen, and Kupffer cells and sinusoidal epithelial cells in the liver. Mycobacterial 16S ribosomal RNA was also isolated, demonstrating that the mycobacteria were viable. Thus, in a TB-endemic area, *M. tuberculosis* was found to persist in multiple locations in the majority of persons without known active TB. Interestingly, none of the *M. tuberculosis* organisms demonstrated were associated with granulomas, inflammatory infiltrates or fibrosis. These results challenge the traditional assumption that latent *M. tuberculosis* persists largely, if not exclusively, in isolated granulomatous foci. Moreover, dissemination of *M. tuberculosis* after primary infection is most likely widespread, and the locus of reactivation TB disease may be more dependent on the local immune milieu than on the site of primary infection.

### Clinical pharmacology

The relationship between serum anti-TB drug concentrations and clinical outcomes of TB treatment has puzzled investigators for the past two decades. Patients with serum drug concentrations that are "subtherapeutic" often respond well to treatment, while those with high levels may still fail to convert their sputum cultures. PASIPANODYA *et al.* [6] studied the serum drug concentrations of isoniazid, rifampin and pyrazinamide, and correlated them with TB treatment outcomes of 142 patients with drug-susceptible TB. Substantial variability in serum drug levels was seen among patients with standard drug dosing. Overall, 15 out of 142 patients failed to convert their sputum cultures after 2 months of therapy and 36 had a poor clinical outcome (two failed to convert their sputum cultures by the end of therapy, 19 relapsed and 15 died). Classification and Regression Tree analysis defined area under the curve cut-offs using the data, and showed a modest correlation between having one drug below the threshold and a poor long-term outcome (OR 2.65, 95% CI 0.99–7.18). However, when two or more drugs were below the cut-off, the odds ratio for a poor long-term outcome was 7.57 (95% CI 2.57–22.3). These cut-offs and their correlations with clinical outcomes need to be confirmed prospectively, but they promise to clarify some of the mystery about the relationship between serum drug concentrations and clinical outcomes.

## Genetics

The clinical relevance of strain variation among *M. tuberculosis* isolates has long been a matter of dispute. While some TB strains appear to display differential growth kinetics or virulence *in vitro* and in animal models, these results have not, in general, predicted clinical transmission or disease manifestations. A new study by FORD *et al.* [7] examined strains of the Euro-American and Beijing lineage for genetic clues that might explain the increased rates of emergence of drug resistance that have been observed in Beijing strains. The overall rate of mutations was 0.3–0.5 mutations per genome per year, corroborating the results of ROETZER *et al.* [3]. Evolution of mutations was studied *in vitro* either with or without antibiotic pressure. Beijing family strains had higher mutation rates and a higher frequency of evolution of antibiotic resistance under pressure than Euro-American isolates. These results provide a biological mechanism for the increased frequency of evolution of drug resistance in Beijing family strains.

## Treatment

Another study on the emergence of drug resistance was one of the clinical highlights of 2013, although it was not good news. DALTON *et al.* [8] performed a prospective study of the prevalence of and risk factors for drug resistance among 1278 patients in eight countries (Estonia, Latvia, Peru, the Philippines, Russia, South Africa, South Korea and Thailand) between 2005 and 2008. The authors demonstrated that, in addition to being resistant to isoniazid and rifampin, 49% of patient isolates were also resistant to ethambutol and streptomycin, while 43.7% showed resistance to at least one second-line drug, 12.9% were resistant to fluoroquinolones and 6.7% were extensively drug-resistant (XDR)-TB [8]. The strongest risk factor for XDR-TB was previous treatment with a second-line injectable drug. Other significant risk factors were female sex and not being in a Green Light Committee-approved treatment programme. These results confirm the global nature of the multidrug-resistant (MDR)-TB epidemic and indicate that we continue to manufacture drug resistance at an alarming rate. Moreover, quality approved treatment programmes (at least as assessed by Green Light Committee approval) had 55% lower rates of XDR-TB.

A clinical trial of linezolid for patients with XDR-TB was another treatment highlight of the year. LEE *et al.* [9] treated 39 patients with smear-positive XDR-TB with either 300 mg per day or 600 mg per day of linezolid in addition to an optimised regime of third-line drugs in an immediate *versus* delayed treatment design. The immediate treatment group had a remarkable 79% sputum culture conversion, compared with 35% in the delayed therapy arm ( $p=0.001$ ). However, 82% of patients had clinically significant adverse events attributed to linezolid (four had dose reduction and three discontinued therapy), and isolates from four out of 39 patients developed resistance to linezolid. These results demonstrate that linezolid, while relatively toxic, may have a role to play in the treatment of XDR-TB and, possibly, some cases of MDR-TB. How to best use the drug remains to be determined. Clearly, the addition of linezolid to an already ineffective regimen quickly generates drug resistance and is not recommended.

A second study also focused on the efficacy and toxicity of linezolid in MDR-TB treatment. The individual patient meta-analysis of SOTGIU *et al.* [10] describes experience with linezolid as part of a multidrug regimen in 121 patients with MDR-TB. Clinical responses were encouraging (99 out of 121 subjects had successful outcomes), but toxicity was substantial. 59% of patients had adverse events, and 54 such events were serious. These investigators saw increased rates of adverse events when  $>600$  mg per day was given. Clearly, linezolid is not a first-line drug for treatment of MDR-TB, and further studies are needed to identify the best ways to use this drug effectively to cure XDR-TB.

An exciting new antimycobacterial agent, PA-824, was studied in an early bactericidal activity study by DIACON *et al.* [11]. Combinations of PA-824 with pyrazinamide, PA-824 with moxifloxacin plus pyrazinamide and PA-824 plus bedaquiline were compared to bedaquiline alone, bedaquiline plus pyrazinamide, or standard HRZE (isoniazid (H), rifampin (R), pyrazinamide (Z) and ethambutol (E)). The combination of PA-824, moxifloxacin and pyrazinamide had the best activity and may be an attractive new regimen for TB treatment.

Long-term outcomes of another new antimycobacterial agent, delamanid, were also reported in 2013. SKRIPCIONKA *et al.* [12] described the follow-up of patients treated in the previously reported randomised, placebo-controlled trial of delamanid for 8 weeks in the initiation phase of treatment for MDR-TB. Favourable outcomes were reported in 75% of patients who received delamanid for  $\geq 6$  months, compared with favourable outcomes in 55% patients who received delamanid for  $\leq 2$  months. While the randomisation of the original study was not maintained, this study adds to our knowledge of the tolerability and efficacy of delamanid in the treatment of MDR-TB.

Two additional studies from the individual patient meta-analysis of patients with MDR-TB were published in 2013, and provide important insights [13, 14]. These reports confirm the poor prognosis of patients whose *M. tuberculosis* isolates are resistant to fluoroquinolones. Among patients with XDR-TB, treatment

outcomes were better when patients received at least six drugs in the intensive phase and four in the continuation phase. These studies also confirmed that patients with XDR-TB with resistance beyond that to second-line injectables and fluoroquinolones experienced worse outcomes than XDR-TB patients without resistance to additional group 4 drugs.

### Prevention

Finally, an important TB vaccination trial was reported in 2013. The MVA85A vaccine, a modified vaccinia Ankara virus expressing *M. tuberculosis* antigen 85A, had shown protection in animal models and promising immunogenicity in human studies when given to previously bacille Calmette–Guérin vaccinated infants. Therefore, a phase 2b trial was initiated to assess efficacy. The results, reported by Tameris *et al.* [15], were disappointing. 32 out of 1399 MV85A recipients developed TB, compared to 39 out of 1395 controls, a vaccine efficacy of 17.3%. The vaccine was well-tolerated and induced “modest” cell-mediated immunity. Not only have hopes of having an effective TB vaccine available soon been deflated, but these results raise questions about the correct immunological response to target in TB vaccine development.

### Conclusions

These reports represent the cutting edge of TB clinical and basic science, and are evidence of a resurgence in high-quality TB research. While not all of the studies gave the results we would like to have seen, taken together, they provide important insights for the next steps towards control and eventual elimination of TB.

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