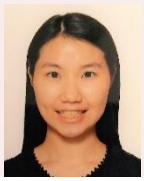
The Road Ahead is Still Tough!

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Notable advances in tuberculosis (TB) diagnostic technologies and TB drugs with a novel mechanism of action have been made in the past several years.[1,2] Potential exists for translating these developments into meaningful improvements in global TB clinical care and control. Notwithstanding, current tools and strategies for diagnosis and treatment of TB are still inadequate. We report a case with delayed diagnosis of extensively drug-resistant TB (XDR-TB), highlighting the difficulties we still face in the management of this disease.

Case History

The patient is a female, aged 28, non-Chinese Hong Kong resident. She moved to Hong Kong from a country in South Asia in recent years. She enjoyed good past health before. She was admitted to an acute hospital on 16th April 2016 for on and off fever and cough for one month. Prior to that she had received two courses of antibiotics from a general practitioner but her symptoms did not improve.

Physical examination was unremarkable. Investigation showed a normal white blood cell count, but the erythrocyte sedimentation rate (ESR) was raised to 71 mm/h, while the C-reactive protein (CRP) was 50 mg/L. Anti-HIV antibody and hepatitis B surface antigen were negative. Chest radiography showed consolidation in right upper and left lower zones (Figure 1). Sputum smear examination for acid fast bacilli (AFB) was positive and Mycobacterium tuberculosis polymerase chain reaction (MTB PCR) was also positive. Genotypic susceptibility test was not done. Anti-TB treatment was started on 18th April 2016 with isoniazid, rifampicin, ethambutol and pyrazinamide. Her fever improved. She was discharged from hospital after 2 weeks and referred to chest clinic for continuation of treatment under directly observed therapy.

The patient's chest radiograph showed minimal improvement in right upper zone but not left lower zone at 5 weeks after initiation of first line anti-TB treatment (Figure 2). Pre-treatment sputum culture result became available at about 8 weeks, and showed MTB organisms resistant to isoniazid, rifampicin, streptomycin and

ethambutol. Molecular line probe assay showed mutation in katG gene (associated with isoniazid resistance), rpoB gene (associated with rifampicin resistance) and rrs gene (associated with aminoglycosides and cyclic peptide resistance). Mutation in gyrA and gyrB genes (associated with resistance to fluoroquinolones) was not detected.

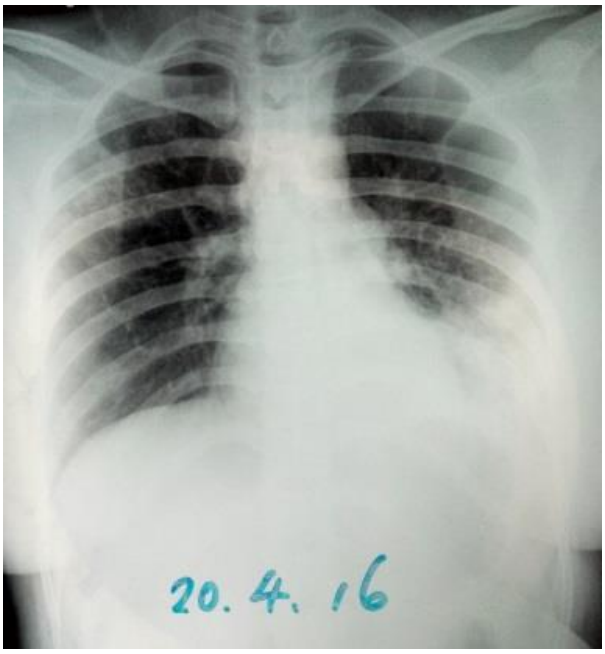

Figure 1. CXR at around start of anti-TB treatment

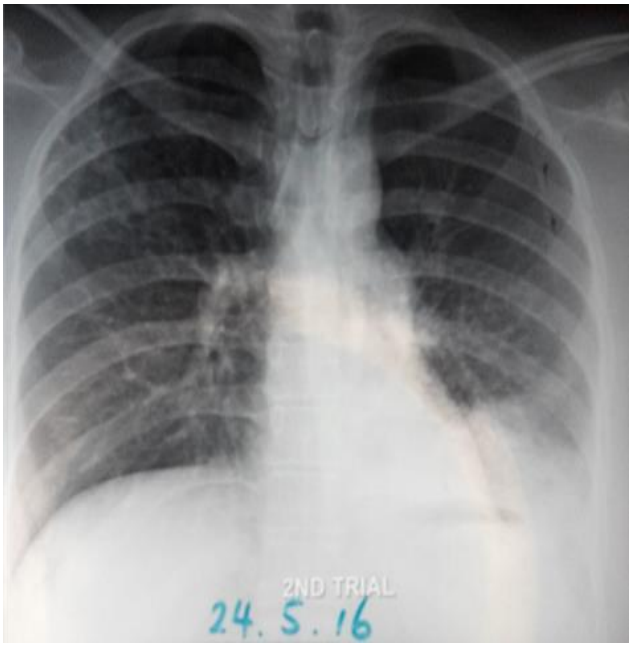

Figure 2. CXR at about 5 weeks of starting first line drugs.

The patient was admitted to chest hospital on 10th June 2016. Sputum AFB smear examination was negative on 12th June 2016 but became positive again on 22nd June 2016. Chest radiograph showed persistent left lower zone shadow. Treatment with second line anti-TB drugs was initiated after admission (on 10th June 2016) for multi-drug resistant (MDR) TB. The regimen comprised kanamycin, prothionamide, cycloserine, levofloxacin, pyrazinamide and linezolid. Pyridoxine was also prescribed. She was discharged home after about 2 weeks. She continued to receive anti-TB treatment at chest clinic subsequently and experienced gastro-intestinal upset and decreased appetite leading to transient interruptions of treatment. Her complete blood count, renal and liver function remained normal.

At 7 week after initiation of second line anti-TB treatment, the pre-treatment sputum culture/drug susceptibility test (DST) result became available and showed bacillary resistance to fluoroquinolones in addition to

resistance to isoniazid, rifampcin and second line injection drugs, i.e. the patient had XDR-TB phenotypically despite gyrA & gyrB mutation were not detected by molecular line probe assay performed earlier. Further DST showed resistance to prothionamide and pyrazinamide as well. Linezolid remained the only effective drug among all the TB drugs tested. On the contrary, the patient's chest and systemic symptoms improved. The ESR improved from 60 to 31 mm/h. Sputum AFB smear examination was scanty positive. Chest radiograph repeated on 16th Aug 2016 (at about 2 month of second line anti-TB treatment) showed improvement in left lower zone shadow (Figure 3).

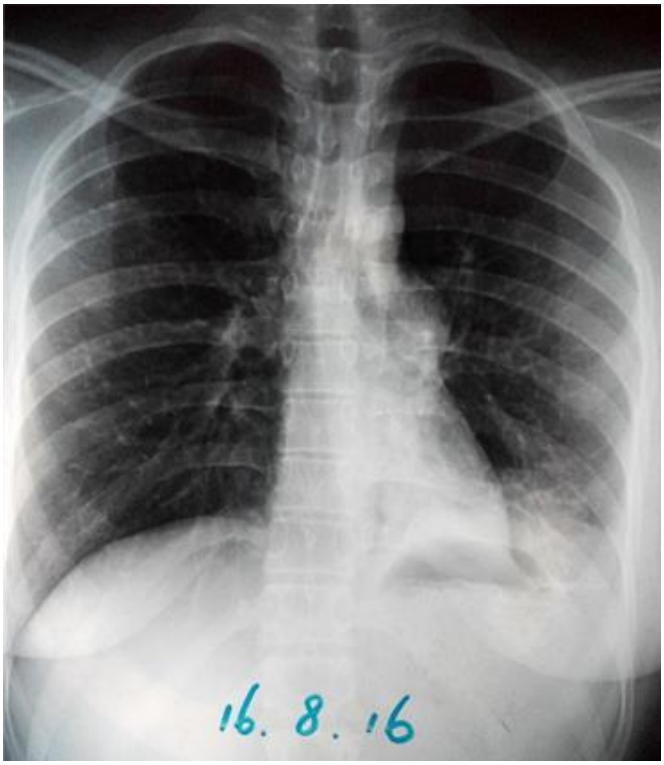

Figure 3. CXR after 2 month treatment with prothionamide, levofloxacin, cycloserine, pyrazinamide, linezolid

In view of the extensively drug resistant pattern and that there were insufficient companion drugs to protect linezolid, the regimen was changed to delamanid, linezolid, levofloxacin, cycloserine and para-aminosalicylic acid (bedaquiline was not available yet in Hong Kong at that time). There was no evidence of acquired resistance to linezolid or treatment failure at the time the regimen was changed. The patient's progress was satisfactory thereafter, and her sputum AFB smear and culture remained consistently negative. Chest radiograph repeated at about 2 and 6 month of switching to delamanid-containing regimen

showed further improvement in left lower zone shadow (Figure 4 & 5). The patient tolerated anti-TB treatment fairly well, except for pin-prick sensation over both legs, which improved with readjusting the dosage interval of linezolid from 600mg daily 6 times per week to 5 times and then 3 times per week, as well as increasing the dose of pyridoxine to 100 mg daily. Her complete blood count, liver and renal function remained normal. Her corrected QT (QTc) interval remained normal, ranging from 433-471 millisecond at chest clinic. It was intended to continue treatment with a delamanid and linezolid containing regimen for at least one year if the patient could tolerate.

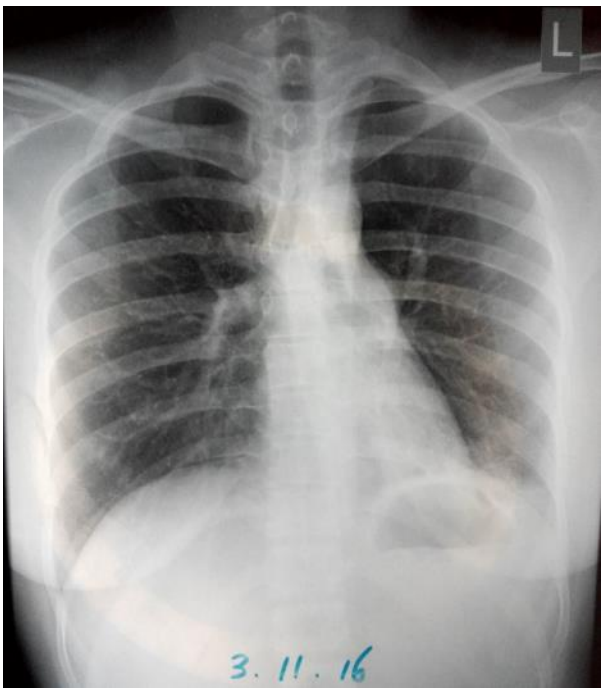

Figure 4. CXR about 2 month after initiation of new regimen containing both delamanid and linezolid

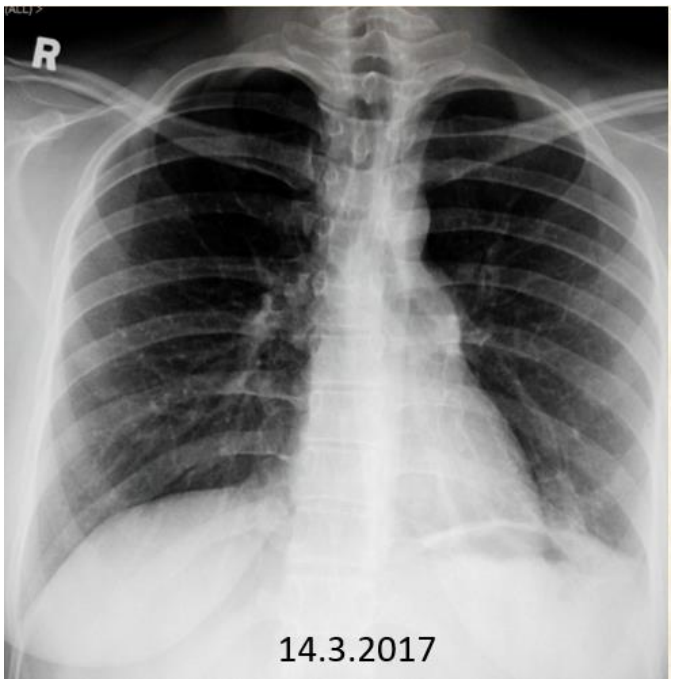

Figure 5. CXR about 6 months after initiation of new regimen containing both delamanid and linezolid

Discussion

Management of drug-resistant TB, especially MDR- and XDR-TB, poses a great challenge to TB health care workers. Rapid, accurate diagnosis is crucial for timely initiation of anti-TB treatment. In this regard, it is encouraging that several new diagnostics have rolled out and that the TB diagnostics landscape has dramatically changed in the past decade. Cartridge-based nucleic acid amplification tests such as the Xpert MTB/RIF assay has been endorsed by WHO as an initial diagnostic test

for TB followed by DST for second line anti-TB agents when necessary.[3] The assay can also rapidly detect rifampicin resistance with high accuracy. Line probe assays (LPAs), such as the commercially available GenoType MTBDRplus assay (Hain Lifescience, Nehren, Germany), and the Nipro NTM+MDRTB detection kit 2 (Nipro, Tokyo,
Japan), which detect mutations associated with drug resistance to rifampicin and isoniazid, have likewise been recommended by WHO for testing sputum smear-positive specimens (direct testing) and cultured isolates of MTB complex (indirect testing) from both pulmonary and extra-pulmonary sites. For detection of resistance to second-line drugs, WHO recommends that the GenoType MTBDR*sl* assay may be used as the initial test instead of phenotypic culture-based DST to detect resistance to fluoroquinolones (mutations in gyrA and gyrB quinolone resistance-determining regions) and to kanamycin, amikacin and capreomycin (mutations in the rrs and eis genes).[4]

Apart from advances in molecular tests for detecting genotypic drug resistance, the past decade has also witnessed the advent of microtiter plates containing lyophilized anti-mycobacterials configured for determination of minimum inhibitory concentrations (MICs) to first- and second-line anti-TB drugs such as The Trek Sensititre MYCOTB MIC plate (MYCOTB; Trek Diagnostic Systems, Cleveland, OH).[5] These tests eliminate the need for in-laboratory preparation (and maintenance) of drug stocks and solutions, which can be a source of variability over time and between laboratories. Agreement between MYCOTB and agar proportion method with respect to susceptibility or resistance was high for most drugs, but was lower for moxifloxacin, ethambutol, cycloserine, and ethionamide.[5] Improving the sensitivity and specificity of DST by enhancing the MYCOTB panel specifically for these drugs will need to be further explored.

While significant progress has been achieved in the area of new TB diagnostics, the development of new anti-TB drugs has been relatively slow. After forty years without newly approved drug classes, TB treatment has recently advanced with the approval of two new drugs for treating MDR-TB: delamanid and bedaquiline. Delamanid is a nitroimidazopyran that works by inhibiting mycolic acid biosynthesis. It is prescribed at a dose of 100 mg twice daily for 24 weeks. In a randomized, placebo-controlled, multinational clinical trial conducted by Gler MT et al, patients who received a background drug regimen plus 100 mg of delamanid twice daily had a higher sputum culture conversion rate at 2 months compared to patients who received a background drug regimen plus placebo (45.4%

vs 29.6%, P = 0.008).[6] In an open label extension of the trial that evaluated delamanid in combination with optimized background regimen for an additional 6 months, favourable outcomes were observed in 143/192 patients (74.5%) who received delamanid for ≥6 months, compared to 126/229 patients (55.0%) who received delamanid for ≤2 months. Mortality was reduced to 1.0% among those receiving long-term delamanid, versus short-term/no delamanid (8.3%), p<0.001.[7] Potential QTc interval prolongation associated with use of delamanid, however, is a concern. In the study by Gler MT, patients who received delamanid plus the background drug regimen had more episodes of QTc interval prolongation on scheduled ECG, as compared with those who received placebo plus the background drug regimen, though none of these episodes were associated with clinical manifestations such as syncope or arrhythmias.[6]

Bedaquiline is a diarylquinoline that acts on mycobacteria by depleting intracellular adenosine triphosphate (ATP) through inhibition of mycobacteria ATP synthase. As a result of a long half-life of 5 months, bedaquiline is prescribed at 400 mg once daily for 2 weeks, followed by 200 mg 3 times per week, for a total of 24 weeks. In a randomized, double-blind, placebo-controlled study conducted by Diacon AH, bedaquiline reduced the median time to sputum culture conversion, as compared with placebo, from 125 days to 83 days (hazard ratio in the bedaquiline group, 2.44; 95% confidence interval, 1.57 to 3.80; P<0.001) and increased the rate of culture conversion at 24 weeks (79% vs. 58%, P = 0.008) and at 120 weeks (62% vs. 44%, P = 0.04).[8] The efficacy and safety of bedaquiline in the treatment of MDR-TB has been aptly summarized in a recent editorial published in Eur Respir J.[9] The evidence of the studies evaluated in that article seems to suggest that bedaquiline is a safe and effective drug. Nonetheless information about cardiac safety of bedaquiline in association with delamanid, clofazimine or fluoroquinolones use is still scanty. Combined use of delamanid and bedaquiline is not currently recommended by WHO, though criteria have been proposed to identify patients and settings where such a combination might be administered.[10] Of note, hepatotoxicity may also occur with the use of bedaquiline.[8]

In addition to the above-mentioned novel anti-TB drugs, repurposed drugs, namely linezolid and clofazimine, have been used to treat patients with MDR- and XDR-TB when there are inadequate first and second line anti-TB drugs and an effective regimen cannot be composed. In a study conducted by Lee M et al, 41 patients with refractory XDR-TB were randomly assigned to linezolid therapy that started immediately or after 2 months, at a dose of 600 mg per day, without a change in their

background regimen. By 4 months, 15 of the 19 patients (79%) in the immediate-start group and 7 of the 20 (35%) in the delayed-start group had culture conversion (P = 0.001).[11] Linezolid has become virtually essential in the treatment of fluoroquinolone-resistant MDR-TB and XDR-TB. Prolonged use of linezolid, however, is hampered by bone marrow suppression and peripheral neuropathy through inhibition of mitochondrial protein synthesis.[12] The adverse events can be reduced by decreasing dosage and/or increasing dosing intervals of linezolid.[13] Clofazimine, another repurposed drug, when added to gatifloxacin, ethambutol, and pyrazinamide given for at least 9 months supplemented by prothionamide, kanamycin, and high-dose isoniazid during an intensive phase of a minimum of 4 months, has been shown to result in a relapse-free cure of 87.9% (95% CI 82.7–91.6) among patients with MDR-TB previously untreated with second line drugs in a Bangladesh prospective observational study.[14] In a systematic review of clofazimine for the treatment of drug-resistant TB, 65% (95%CI 52–79) of MDR-TB and 66% (95%CI 42–89) of XDR-TB experienced favorable treatment outcomes using random effects meta-analysis.[15] Nonetheless, prospective cohort studies and clinical trials examining the effect of clofazimine as part of drug-resistant TB treatment regimens are needed.

With the advent of novel and repurposed anti-TB drugs, and based on reviews of aggregated and individual patient data from published and unpublished studies, WHO has updated its guideline on treatment of drug-resistant TB in 2016.[16] Drugs recommended for treatment of MDR-TB are classified into 4 groups (Group A to Group D, Table 1), and details are given on the general steps in designing the composition of a longer MDR-TB regimen. Nonetheless, the 2016 revision highlighted the continued shortage of high-quality evidence and implementation research, and reiterated the need for clinical trials and best-practice studies to improve MDR-TB patient treatment outcomes and strengthen policy.

Group	**Description**	**Drugs**
A	Fluoroquinolones	Levofloxacin, moxifloxacin, gatifloxacin
B	Second-line injectable agents	Kanamycin, Amikacin, capreomycin
C	Other core agents	Ethionamide or prothionamide, cycloserine or terizidone, linezolid, clofazimine

D1	Add-on agents: first-line drugs	Pyrazinamide, ethambutol, high-dose isoniazid
D2	Add-on agents: novel drugs	Bedaquiline, delamanid
D3	Add-on agents: others	Para-aminosalicylic acid, imipenem-cilastatin with clavulanate, meropenem with clavulanate, amoxicillin with clavulanate, thiocetazone

Table 1. Drugs recommended by WHO for the treatment of RR-TB and MDR-TB[16]

The timely initiation of effective anti-TB treatment for MDR-TB relies heavily on prompt, accurate diagnosis of the disease. One major challenge in the management of MDR-TB is the occurrence of borderline and low level rifampicin resistance which may be missed by growth-based DST methods, particularly automated broth-based systems. In a study that examined 153 consecutive clinical MTB strains diagnosed as resistant to rifampin with molecular tests in a laboratory in Haiti, genotypic resistance to rifampicin was not confirmed by phenotypic DST in 10.5% of TB cases.[17]

Five strains with discordant genotypic and phenotypic susceptibility results had rifampicin MIC close to the cut-off value of 1 mg/ml used in phenotypic susceptibility assays and 9 strains had sub-critical rifampicin MICs ranging from 0.063 to 0.5 mg/ml. While data on the clinical relevance of low-level rifampicin resistance is scare, borderline resistance to rifampicin has been strongly associated with treatment failure in the literature, suggesting a need to re-evaluate the present critical concentration of the drug used in culture-based DST assays.

Fluoroquinolones have been considered the pivotal drugs in the management of MDR-TB. Detection of fluoroquinolone resistance mutations by rapid molecular tests greatly aid physicians in the formulation of appropriate TB regimen. Fluoroquinolone resistance in MTB can be conferred by mutations in gyrA or gyrB. The prevalence of resistance mutations outside the quinolone resistance-determining region (QRDR) of gyrA or gyrB is, however, unclear as such regions are rarely sequenced. In one study by Tennessee Department of Health, of 25 ofloxacin-resistant isolates, 11 (44%) did not have previously reported resistance mutations.[18] Of these, 10 had novel polymorphisms: 3 in the QRDR of gyrA, 1 in the QRDR of gyrB, and 6 outside the QRDR of gyrA or gyrB; 1 did not have any gyrase polymorphisms.[18] In our case, conventional culture/DST showed bacillary resistance to fluoroquinolones, while gyrA &

gyrB mutations were not detected by molecular line probe assay, leading to a delayed diagnosis of XDR-TB. Mutations outside the QRDR of gyrA or gyrB was a possibility, while hetero-resistance i.e. resistance caused by gyrase mutations that were initially present only in a minority of bacilli too small to be detected by the molecular tests was also possible. More research into fluoroquinolone resistance mutations are definitely needed, as well as alternative fluoroquinolone resistance mechanisms such as efflux pumps, pentapeptide proteins, or enzymes that inactivate the fluoroquinolones.[19]

Conclusion

For the first time in over 40 years, two new TB drugs with a novel mechanism of action – bedaquiline and delamanid - are available. Notable advances in TB diagnostic technologies have also been made in the past several years. Notwithstanding, current tools and strategies for diagnosis of TB are still inadequate, as exemplified by the delayed diagnosis of XDR-TB in the present case. More experience with the use of delamanid and bedaquiline is also needed, especially on the simultaneous use of these two drugs. Results from clinical trials NCT02583048 and NCT02754765 may shed more light on this combination and any possible major interactions. Judicious and rational use of the novel drugs is mandatory to protect these precious drugs, especially in light of a recent report reporting acquired resistance to delamanid and bedaquiline in a patient receiving anti-TB treatment for XDR-TB.[20] More novel drugs for treatment of MDR- and XDR-TB are needed.

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