PubMed Open Access Articles

1. **Trends and challenges of multi-drug resistance in childhood tuberculosis.**

Front Cell Infect Microbiol. 2023 Jun 2;13:1183590. doi: 10.3389/fcimb.2023.1183590. eCollection 2023.

Zhuang Z(1)(2), Sun L(3), Song X(4), Zhu H(1)(2), Li L(1)(5), Zhou X(1), Mi K(1)(2)(4).

Drug-resistant tuberculosis (DR-TB) in children is a growing global health concern, This review provides an overview of the current epidemiology of childhood TB and DR-TB, including prevalence, incidence, and mortality. We discuss the challenges in diagnosing TB and DR-TB in children and the limitations of current diagnostic tools. We summarize the challenges associated with treating multi-drug resistance TB in childhood, including limitations of current treatment options, drug adverse effects, prolonged regimens, and managing and monitoring during treatment. We highlight the urgent need for improved diagnosis and treatment of DR-TB in children. The treatment of children with multidrug-resistant tuberculosis will be expanded to include the evaluation of new drugs or new combinations of drugs. Basic research is needed to support the technological development of biomarkers to assess the phase of therapy, as well as the urgent need for improved diagnostic and treatment options.

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PMCID: PMC10275406
PMID: 37333849 [Indexed for MEDLINE]

Conflict of interest statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

2. **Global treatment outcomes of extensively drug-resistant tuberculosis in adults: A systematic review and meta-analysis.**

J Infect. 2023 Jun 23:S0163-4453(23)00337-7. doi: 10.1016/j.jinf.2023.06.014. Online ahead of print.

Pedersen OS(1), Holmgaard FB(2), Mikkelsen MKD(2), Lange C(3), Sotgiu G(4), Lillebaek T(5), Andersen AB(6), Wejse CM(7), Dahl VN(8).

INTRODUCTION: Historically, extensively drug-resistant tuberculosis has been notoriously difficult to treat with devasting outcomes. As we are coming to the end of an era where the 2006 extensively drug-resistant tuberculosis definitions and old treatment regimens are being replaced, we aimed to estimate the proportion of extensively drug-resistant tuberculosis patients globally who achieved successful treatment outcomes.
METHODS: We conducted a systematic review of PubMed/MEDLINE, Scopus, Web of Science, and Embase from January 1, 2005, through April 3, 2023. Included studies reported WHO treatment outcomes, or adaptions hereof, for pre-extensively and/or extensively drug-resistant tuberculosis patients according to the 2006 WHO definition. Eligible studies included cohorts of at least 10 adults (aged >18 years) that were not pregnant. Using a random-effects model, we calculated pooled proportions of treatment outcomes and performed sensitivity and subgroup analyses. PROSPERO registration number: CRD42022340961.
RESULTS: Among 5,056 studies reviewed, we identified 94 studies from 26 countries, involving 10,223 extensively drug-resistant tuberculosis patients. The pooled proportion of successful treatment outcomes was 44.2% (95%CI: 38.3-50.3). Sensitivity analyses consistently produced similar estimates. A slight improvement in treatment outcomes was observed after 2013. Furthermore, 25 studies reported outcomes for 3,564 individuals with pre-extensively drug-resistant tuberculosis, of which 63.3% achieved successful treatment (95%CI: 43.1-72.5).
CONCLUSION: Globally, the success rate of extensively drug-resistant tuberculosis treatment is 44.2%, far below the WHO's target rate of 75%. These results may serve as a reference for future studies assessing extensively drug-resistant tuberculosis treatment outcomes under the 2021 definition treated with better treatment regimens available. Comprehensive surveillance data of extensively drug-resistant tuberculosis outcomes from the whole world are desirable to monitor treatment progress.

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PMID: 37356629

Conflict of interest statement: Declaration of Competing Interest CL reports support for the present manuscript (e.g., funding and medical writing) from DZIF (German Center of Infection Research); consulting fees from a consultation service to Insmed, a company that produced liposomal amikacin as an inhalative suspension for the treatment of non-tuberculous mycobacteria pulmonary disease (outside of the scope of this work); speakers' honoraria from Insmed, Gilead, and Janssen (all outside of the scope of this work); is a member of the data safety board of trials from Médecins Sans Frontières (outside of the scope of this work); is supported by the German Center for Infection Research (DZIF); and

acknowledges funding from the European Commission (anTBiotic EU-H2020 733079, ClicTB EDCTP2 RIA2017T-2030, stool4TB EDCTP2 RIAD2018-2511, and UNITE4TB EU-IMI 101007873). ABA and VND are members of the advisory board for Nordicinfu Care Denmark who distributes ARIKAYCE® (amikacin liposome inhalation suspension) for Insmed (outside of the scope of this work). All other authors declare no competing interests.

3. **Long-term treatment outcomes in patients with multidrug-resistant tuberculosis.**

Clin Microbiol Infect. 2023 Jun;29(6):751-757. doi: 10.1016/j.cmi.2023.02.013. Epub 2023 Feb 25.

Maier C(1), Chesov D(2), Schaub D(1), Kalsdorf B(1), Andres S(3), Friesen I(3), Reimann M(1), Lange C(4).

OBJECTIVES: To describe long-term treatment outcomes in patients with multi-drug-resistant/rifampicin-resistant tuberculosis (MDR/RR-TB) and validate established outcome definitions for MDR/RR-TB treatment.
METHODS: Among patients with MDR/RR-TB admitted to a German MDR/RR-TB referral centre from 1 September 2002 to 29 February 2020, we compared long-term treatment outcomes derived from individual patient follow-up with treatment outcomes defined by WHO-2013, WHO-2021 and the Tuberculosis Network European Trials Group-2016.
RESULTS: In a total of 163 patients (mean age, 35 years; standard deviation, 13 years; 14/163 [8.6%] living with HIV; 109/163 [66.9%] men, 149/163 [91.4%] migrating to Germany within 5 years), the treatment of culture-confirmed MDR/RR-TB was initiated. Additional drug resistance to a fluoroquinolone or a second-line injectable agent was present in 15 of the 163 (9.2%) Mycobacterium tuberculosis strains; resistance against both the drug classes was present in 29 of the 163 (17.8%) strains. The median duration of MDR/RR-TB treatment was 20 months (interquartile range, 19.3-21.6 months), with a medium of five active drugs included. The median follow-up time was 4 years (47.7 months; interquartile range, 21.7-65.8 months). Among the 163 patients, cure was achieved in 25 (15.3%), 82 (50.3%) and 95 (58.3%) patients according to the outcome definitions of WHO-2013, WHO-2021, and the Tuberculosis Network European Trials Group-2016, respectively. The lost to follow-up rate was 17 of 163 (10.4%). Death was more likely in patients living with HIV (hazard ratio, 4.28; 95% confidence interval, 1.26-12.86) and older patients (hazard ratio, 1.08; 95% confidence interval, 1.05-1.12; increment of 1 year). Overall, 101/163 (62.0%) patients experienced long-term, relapse-free cure; of those, 101/122 (82.8%) patients with a known status (not lost to-follow-up or transferred out) at follow-up.
CONCLUSION: Under optimal management conditions leveraging individualized

treatment regimens, long-term, relapse-free cure from MDR/RR-TB is substantially higher than cure rates defined by current treatment outcome definitions.

DOI: 10.1016/j.cmi.2023.02.013
PMID: 36842637 [Indexed for MEDLINE]

4. **Outcomes of WHO-conforming, longer, all-oral multidrug-resistant TB regimens and analysis implications.**

Int J Tuberc Lung Dis. 2023 Jun 1;27(6):451-457. doi: 10.5588/ijtld.22.0613.

Rich ML(1), Khan U(2), Zeng C(3), LaHood A(3), Franke MF(3), Atwood S(4), Bastard M(5), Burhan E(6), Danielyan N(7), Dzhazibekova PM(8), Gadissa D(9), Ghafoor A(10), Hewison C(11), Islam MS(12), Kazmi E(13), Khan PY(14), Lecca L(15), Maama LB(16), Melikyan N(17), Naing YY(18), Philippe K(19), Saki NA(20), Seung KJ(1), Skrahina A(21), Tefera GB(9), Varaine F(11), Vilbrun SC(22), Võ L(23), Mitnick CD(24), Huerga H(5).

BACKGROUND: Evidence of the effectiveness of the WHO-recommended design of longer individualized regimens for multidrug- or rifampicin-resistant TB (MDR/RR-TB) is limited.OBJECTIVES: To report end-of-treatment outcomes for MDR/RR-TB patients from a 2015-2018 multi-country cohort that received a regimen consistent with current 2022 WHO updated recommendations and describe the complexities of comparing regimens.METHODS: We analyzed a subset of participants from the endTB Observational Study who initiated a longer MDR/RR-TB regimen that was consistent with subsequent 2022 WHO guidance on regimen design for longer treatments. We excluded individuals who received an injectable agent or who received fewer than four likely effective drugs.RESULTS: Of the 759 participants analyzed, 607 (80.0%, 95% CI 77.0-82.7) experienced successful end-of-treatment outcomes. The frequency of success was high across groups, whether stratified on number of Group A drugs or fluoroquinolone resistance, and ranged from 72.1% to 90.0%. Regimens were highly variable regarding composition and the duration of individual drugs.CONCLUSIONS: Longer, all-oral, individualized regimens that were consistent with 2022 WHO guidance on regimen design had high frequencies of treatment success. Heterogeneous regimen compositions and drug durations precluded meaningful comparisons. Future research should examine which combinations of drugs maximize safety/tolerability and effectiveness.

DOI: 10.5588/ijtld.22.0613
PMCID: PMC10237267
PMID: 37231598 [Indexed for MEDLINE]

Conflict of interest statement: Conflicts of interest: none declared.

5. **Gated Calcium Ion Channel and Mutation Mechanisms in Multidrug-Resistant Tuberculosis.**

Int J Mol Sci. 2023 Jun 2;24(11):9670. doi: 10.3390/ijms24119670.

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A wide spectrum of Gram-positive/Gram-negative bacteria has been found resistant to a wide spectrum of antibiotics in the United States of America during the past decade. Drug-resistant tuberculosis is not yet a major threat in North/South America, Europe, and the Middle East. However, the migration of populations in times of drought, famine, and hostilities may increase the global reach of this ancient pathogen. Given an increased spread from China and India to African countries, drug-resistant Mycobacterium tuberculosis has become an emerging topic of concern for Europe and North America. Due to the dangers associated with the spread of pathogens among different populations, the World Health Organization continues to expand healthcare advisories for therapeutic approaches for both stationary and migrating populations. As much of the literature focuses on endemic to pandemic viruses, we remain concerned that other treatable communicable diseases may be ignored. One such disease is multidrug-resistant tuberculosis. We focus on molecular mechanisms that this pathogen relies upon for the development of multidrug resistance via gene mutation and the evolutionary development of new enzyme and calcium channels.

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PMCID: PMC10253542
PMID: 37298620 [Indexed for MEDLINE]

Conflict of interest statement: The authors declare no conflict of interest.

6. **"Weighting" the Evidence: How Much Bedaquiline Is Enough?**

Am J Respir Crit Care Med. 2023 Jun 1;207(11):1423-1424. doi: 10.1164/rccm.202303-0373ED.

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Comment on
Am J Respir Crit Care Med. 2023 Jun 1;207(11):1525-1532.

DOI: 10.1164/rccm.202303-0373ED
PMCID: PMC10263129
PMID: 37043826 [Indexed for MEDLINE]

7. **Resistance patterns among drug-resistant tuberculosis patients and trends-over-time analysis of national surveillance data in Gabon, Central Africa.**

Infection. 2023 Jun;51(3):697-704. doi: 10.1007/s15010-022-01941-5. Epub 2022 Oct 28.

Abdul JBPAA(#)(1), Adegbite BR(#)(2)(3)(4), Ndanga MED(1), Edoa JR(1), Mevyann RC(1), Mfoumbi GRAI(1), de Dieu TJ(5), Mahoumbou J(6), Biyogho CM(1), Jeyaraj S(7), Niemann S(8), Lell B(1)(5), Kremsner PG(1)(5), Alabi AS(1)(9), Adegnika AA(1)(9)(10)(11), Grobusch MP(12)(13)(14)(15)(16).

OBJECTIVE: Routinely generated surveillance data are important for monitoring the effectiveness of MDR-TB control strategies. Incidence of rifampicin-resistant tuberculosis (RR-TB) is a key indicator for monitoring MDR-TB.
METHODS: In a longitudinal nationwide retrospective study, 8 years (2014-2021) of sputum samples from presumptively drug-resistant tuberculosis patients from all regions of Gabon were referred to the national tuberculosis reference laboratory. Samples were analysed using GeneXpert MTB/RIF and Genotype MTBDRsl version 2/Line Probe Assay.
RESULTS: Of 3057 sputum samples from presumptive tuberculosis patients, both from local hospital and from referral patients, 334 were RR-TB. The median patient age was 33 years (interquartile range 26-43); one third was newly diagnosed drug-resistant tuberculosis patients; one-third was HIV-positive. The proportion of men with RR-TB was significantly higher than that of women (55% vs 45%; $p < 0.0001$). Patients aged 25-35 years were most affected (32%; 108/334). The cumulative incidence of RR-TB was 17 (95% CI 15-19)/100,000 population over 8 years. The highest incidences were observed in 2020 and 2021. A total of 281

samples were analysed for second-line drug resistance. The proportions of study participants with MDR-TB, pre-XDR-TB and XDR-TB were 90.7% (255/281), 9% (25/281) and 0.3% (1/281), respectively. The most-common mutations in fluoroquinolones resistance isolates was gyrA double mutation gyrA MUT3B and MUT3C (23%; 4/17). Most (64%; 6/8) second-line injectable drugs resistance isolates were characterised by missing both rrs WT2 and MUT2 banding.
CONCLUSION: The increasing incidence of MDR-TB infection in Gabon is alarming. It is highest in the 25-35 years age category. The incidence of MDR-TB infection in treatment-naïve patients calls for case finding and contact tracing strategy improvement.

DOI: 10.1007/s15010-022-01941-5
PMCID: PMC9616411
PMID: 36307576 [Indexed for MEDLINE]

Conflict of interest statement: All authors declare that they have no competing interests to disclose.

8. **Characteristics of TB/HIV Co-Infection and Patterns of Multidrug-Resistance Tuberculosis in the Northwest Amhara, Ethiopia.**

Infect Drug Resist. 2023 Jun 16;16:3829-3845. doi: 10.2147/IDR.S412951. eCollection 2023.

Seid A(1)(2), Girma Y(3), Abebe A(3), Dereb E(3), Kassa M(3), Berhane N(2).

BACKGROUND: Multidrug-resistant tuberculosis (MDR-TB) has continued to be a serious public health threat and significantly challenges global TB control and prevention efforts, where the TB/HIV co-infection epidemic makes the situation much worse. The aim of the study was to determine the determinant factors associated with patterns of MDR-TB among pulmonary TB patients in the Northwest Amhara, Ethiopia.
METHODS: A hospital-based cross-sectional study was conducted from May 2022 to February 2023 in the Northwest Amhara, Ethiopia. Data on the participants' socio-demographics and clinical characteristics were obtained using a pre-tested checklist. Phenotypic susceptibility testing to first-line anti-TB drugs was performed on 180 isolates by automated BD BACTEC MGIT 960 system. Logistic regression analysis was performed to determine the association of risk factors with patterns of MDR-TB. A p-value ≤0.05 was considered statistically significant.
RESULTS: The overall proportion of TB with HIV co-infected cases was 19.8%

(50/252). Culture positivity was confirmed in 203/252 (80.6%) of sputum samples. Among 168 isolates, the DST showed that 119 (70.8%) isolates were pan-susceptible to all first-line drugs and prevalence of any resistance to first-line drugs was 49,168 (29.2%). Among the resistant isolates, 28 (16.7%) were any mono-resistance and 12 (7.1%) were determined to be resistant to MDR-TB. TB with a previous TB treatment (aOR = 6.73, 95% CI: 1.78-25.47, p = 0.005) and HIV co-infected (aOR = 0.252, 95% CI: 0.73-0.875, p = 0.03) were significantly associated with MDR-TB.
CONCLUSION: Higher prevalence of TB and MDR-TB was examined among TB patients in the study area. In the study, history of previous TB treatment was the strongest risk factor MDR-TB infection followed by TB with HIV co-infected cases. Therefore, there is a need of strengthening TB control and prevention programs to reduce the increase of TB incidence, further emergence and transmission of a public health threat of MDR-TB cases.

DOI: 10.2147/IDR.S412951
PMCID: PMC10281285
PMID: 37346368

Conflict of interest statement: The authors have declared that no competing interests exist.

9. **Operationalising targeted next-generation sequencing for routine diagnosis of drug-resistant TB.**

Public Health Action. 2023 Jun 21;13(2):43-49. doi: 10.5588/pha.22.0041.

Iyer A(1), Ndlovu Z(2)(3), Sharma J(1), Mansoor H(1), Bharati M(1), Kolan S(1), Morales M(1), Das M(1), Issakidis P(2), Ferlazzo G(2), Hirani N(4), Joshi A(4), Tipre P(5), Sutar N(5), England K(6).

BACKGROUND: Phenotypic drug susceptibility testing (pDST) for Mycobacterium tuberculosis can take up to 8 weeks, while conventional molecular tests identify a limited set of resistance mutations. Targeted next-generation sequencing (tNGS) offers rapid results for predicting comprehensive drug resistance, and this study sought to explore its operational feasibility within a public health laboratory in Mumbai, India.
METHODS: Pulmonary samples from consenting patients testing Xpert MTB-positive were tested for drug resistance by conventional methods and using tNGS. Laboratory operational and logistical implementation experiences from study team members are shared below.

RESULTS: Of the total number of patients tested, 70% (113/161) had no history of previous TB or treatment; however, 88.2% (n = 142) had rifampicin-resistant/multidrug-resistant TB (RR/MDR-TB). There was a high concordance between resistance predictions of tNGS and pDST for most drugs, with tNGS more accurately identifying resistance overall. tNGS was integrated and adapted into the laboratory workflow; however, batching samples caused significantly longer result turnaround time, fastest at 24 days. Manual DNA extraction caused inefficiencies; thus protocol optimisations were performed. Technical expertise was required for analysis of uncharacterised mutations and interpretation of report templates. tNGS cost per sample was US$230, while for pDST this was US$119.
CONCLUSIONS: Implementation of tNGS is feasible in reference laboratories. It can rapidly identify drug resistance and should be considered as a potential alternative to pDST.

DOI: 10.5588/pha.22.0041
PMCID: PMC10290261
PMID: 37359066

Conflict of interest statement: Conflicts of interest: none declared.

10. **Effectiveness of Bedaquiline Use beyond Six Months in Patients with Multidrug-Resistant Tuberculosis.**

Am J Respir Crit Care Med. 2023 Jun 1;207(11):1525-1532. doi: 10.1164/rccm.202211-2125OC.

Trevisi L(1), Hernán MA(2), Mitnick CD(1)(3)(4), Khan U(5), Seung KJ(1)(3)(4), Rich ML(1)(3)(4), Bastard M(6), Huerga H(6), Melikyan N(6), Atwood SA(3), Avaliani Z(7), Llanos F(8)(9), Manzur-Ul-Alam M(10), Zarli K(11), Binegdie AB(12), Adnan S(13), Melikyan A(14), Gelin A(15), Isani AK(16), Vetushko D(17), Daugarina Z(18), Nkundanyirazo P(19), Putri FA(5), Vilbrun C(20), Khan M(21), Hewison C(22), Khan PY(23), Franke MF(1).

Comment in
Am J Respir Crit Care Med. 2023 Jun 1;207(11):1423-1424.

Rationale: Current recommendations for the treatment of rifampicin- and multidrug-resistant tuberculosis include bedaquiline (BDQ) used for 6 months or longer. Evidence is needed to inform the optimal duration of BDQ. Objectives: We emulated a target trial to estimate the effect of three BDQ duration treatment

strategies (6, 7-11, and ⩾12 mo) on the probability of successful treatment among patients receiving a longer individualized regimen for multidrug-resistant tuberculosis. Methods: To estimate the probability of successful treatment, we implemented a three-step approach comprising cloning, censoring, and inverse probability weighting. Measurements and Main Results: The 1,468 eligible individuals received a median of 4 (interquartile range, 4-5) likely effective drugs. In 87.1% and 77.7% of participants, this included linezolid and clofazimine, respectively. The adjusted probability of successful treatment was 0.85 (95% confidence interval [CI], 0.81-0.88) for 6 months of BDQ, 0.77 (95% CI, 0.73-0.81) for 7-11 months, and 0.86 (95% CI, 0.83-0.88) for ⩾12 months. Compared with 6 months of BDQ, the ratio of treatment success was 0.91 (95% CI, 0.85-0.96) for 7-11 months and 1.01 (95% CI, 0.96-1.06) for ⩾12 months. Naive analyses that did not account for bias revealed a higher probability of successful treatment with ⩾12 months (ratio, 1.09 [95% CI, 1.05-1.14]). Conclusions: BDQ use beyond 6 months did not increase the probability of successful treatment among patients receiving longer regimens that commonly included new and repurposed drugs. When not properly accounted for, immortal person-time bias can influence estimates of the effects of treatment duration. Future analyses should explore the effect of treatment duration of BDQ and other drugs in subgroups with advanced disease and/or receiving less potent regimens.

DOI: 10.1164/rccm.202211-2125OC
PMCID: PMC10263131
PMID: 36802336 [Indexed for MEDLINE]

11. **Post-tuberculosis lung impairment: systematic review and meta-analysis of spirometry data from 14 621 people.**

Eur Respir Rev. 2023 Apr 19;32(168):220221. doi: 10.1183/16000617.0221-2022. Print 2023 Jun 30.

Ivanova O(1)(2)(3), Hoffmann VS(4)(3), Lange C(5)(6)(7)(8), Hoelscher M(9)(2), Rachow A(9)(2).

BACKGROUND: A substantial proportion of tuberculosis patients remain with pulmonary symptoms and reduced physical capacity despite successful treatment. We performed a systematic review to analyse the burden of post-tuberculosis lung impairment measured by lung function testing.
METHODS: We searched the PubMed database for articles published between database inception and November 2020 and performed meta-analyses to estimate the prevalence, type and severity of lung impairment among drug-susceptible and multidrug-resistant tuberculosis survivors. Methodological quality of included studies was assessed using the Newcastle-Ottawa scale.

RESULTS: 54 articles were included in this review. For subjects with former drug-susceptible tuberculosis, the combined estimated mean was 76.6% (95% CI 71.6-81.6) of predicted for forced expiratory volume in 1 s (FEV1) and 81.8% (95% CI 77.4-86.2) for forced vital capacity (FVC). In former patients with multidrug-resistant tuberculosis, it was 65.9% (95% CI 57.1-74.7) for FEV1 and 76.0% (95% CI 66.3-85.8) for FVC, respectively. The analysis of impairment types in former patients with drug-susceptible and multidrug-resistant tuberculosis showed that 22.0% versus 19.0% had obstructive, 23.0% versus 22.0% restrictive and 15.0% versus 43.0% had mixed impairment type, respectively. In the majority of studies, at least 10-15% of tuberculosis survivors had severe lung impairment.
CONCLUSIONS: This systematic review showed long-term abnormal spirometry results in a significant proportion of tuberculosis survivors.

DOI: 10.1183/16000617.0221-2022
PMCID: PMC10113954
PMID: 37076175 [Indexed for MEDLINE]

Conflict of interest statement: Conflict of interest: The authors declare no conflict of interest.

12. **Optimizing tuberculosis treatment efficacy: Comparing the standard regimen with Moxifloxacin-containing regimens.**

PLoS Comput Biol. 2023 Jun 15;19(6):e1010823. doi: 10.1371/journal.pcbi.1010823. eCollection 2023 Jun.

Budak M(1), Cicchese JM(2), Maiello P(3), Borish HJ(3), White AG(3), Chishti HB(3), Tomko J(3), Frye LJ(3), Fillmore D(3), Kracinovsky K(3), Sakal J(3), Scanga CA(3), Lin PL(3), Dartois V(4)(5), Linderman JJ(2), Flynn JL(3), Kirschner DE(1).

Tuberculosis (TB) continues to be one of the deadliest infectious diseases in the world, causing ~1.5 million deaths every year. The World Health Organization initiated an End TB Strategy that aims to reduce TB-related deaths in 2035 by 95%. Recent research goals have focused on discovering more effective and more patient-friendly antibiotic drug regimens to increase patient compliance and decrease emergence of resistant TB. Moxifloxacin is one promising antibiotic that may improve the current standard regimen by shortening treatment time. Clinical trials and in vivo mouse studies suggest that regimens containing moxifloxacin have better bactericidal activity. However, testing every possible

combination regimen with moxifloxacin either in vivo or clinically is not feasible due to experimental and clinical limitations. To identify better regimens more systematically, we simulated pharmacokinetics/pharmacodynamics of various regimens (with and without moxifloxacin) to evaluate efficacies, and then compared our predictions to both clinical trials and nonhuman primate studies performed herein. We used GranSim, our well-established hybrid agent-based model that simulates granuloma formation and antibiotic treatment, for this task. In addition, we established a multiple-objective optimization pipeline using GranSim to discover optimized regimens based on treatment objectives of interest, i.e., minimizing total drug dosage and lowering time needed to sterilize granulomas. Our approach can efficiently test many regimens and successfully identify optimal regimens to inform pre-clinical studies or clinical trials and ultimately accelerate the TB regimen discovery process.

DOI: 10.1371/journal.pcbi.1010823
PMCID: PMC10306236
PMID: 37319311 [Indexed for MEDLINE]

Conflict of interest statement: None.

13. **Bedaquiline resistance pattern in clofazimine-resistant clinical isolates of tuberculosis patients.**

J Glob Antimicrob Resist. 2023 Jun;33:294-300. doi: 10.1016/j.jgar.2023.04.003. Epub 2023 May 3.

Shang Y(1), Chen S(2), Shi W(3), Nie W(3), Jing W(3), Huo F(2), Xue Y(2), Dong L(2), Jiang G(2), Huang H(4), Chu N(5).

OBJECTIVES: Bedaquiline (BDQ) is a potent drug for treating drug-resistant tuberculosis (TB). Here, we analysed the resistance profiles of BDQ in CFZ-resistant clinical isolates and investigated the clinical risk factors of BDQ and CFZ cross/co-resistance.
METHODS: The AlarmarBlue microplate assay was performed to determine the minimum inhibitory concentration (MIC) of the CFZ-resistant Mycobacterium tuberculosis (MTB) clinical isolates to CFZ and BDQ. The clinical characteristics of the respective patients were analysed to explore the possible risk factors of BDQ resistance. The drug-resistance-associated genes including Rv0678, Rv1979c,

atpE, pepQ and Rv1453 were sequenced and analysed.
RESULTS: A total of 72 clinical CFZ-resistant MTB isolates were collected; among these, half were identified as BDQ-resistant. The MIC value of BDQ closely correlated with CFZ (Spearman's q = 0.766, P < 0.005). Among the isolates with a MIC of CFZ ≥4 mg/L, 92.31% (12/13) were resistant to BDQ. Pre-XDR and exposure to BDQ or CFZ are the major risk factors for concurrent BDQ resistance. Among the 36 cross/co-resistant isolates, 50% (18/36) had mutations in Rv0678, 8.3% (3/36) had mutations in Rv0678+Rv1453, 5.6% (2/36) had mutations in Rv0678+Rv1979c, 2.8% (1/36) had mutations in Rv0678+Rv1979c+Rv1453, 2.8% (1/36) had mutations in atpE+Rv0678+Rv1453, 2.8% (1/36) had mutations in Rv1979c, and 27.7% (10/36) had no variations in the target genes.
CONCLUSION: Nearly half of the CFZ-resistant isolates were still sensitive to BDQ, whereas this rate dramatically decreased among patients with pre-XDR TB or those who had been exposed to BDQ or CFZ.

DOI: 10.1016/j.jgar.2023.04.003
PMID: 37142094 [Indexed for MEDLINE]

14. **Effect of Stenotrophomonas maltophilia on Tuberculosis.**

Microbiol Spectr. 2023 Jun 12:e0094423. doi: 10.1128/spectrum.00944-23. Online ahead of print.

Li Y(1), Zhao A(2), Yu Q(2), Yu N(2), Cui Y(1), Ma X(1), Liu H(1), Wang R(1).

Tuberculosis (TB) is an important infectious disease suffered by many countries, including China. In this stage, accurate diagnosis and treatment are key measures for the prevention and control of TB. Stenotrophomonas maltophilia is a global emerging Gram-negative, multidrug-resistant (MDR) organism characterized by its high contribution to the increase in crude mortality rates. By single cell preparation and strain identification, we isolated S. maltophilia from stored cultures of Mycobacterium tuberculosis (Mtb). We found that S. maltophilia could not be removed from sputum by alkali treatment or inhibited by antibiotic mixture added to MGIT 960 indicator tubes. When co-cultured with Mtb on a Löwenstein-Jensen (L-J) slant, it could inhibit the growth of Mtb and liquefy the medium. More seriously, it was resistant to 10 of the 12 anti-TB drugs, including isoniazid and rifampin, and made the mixed samples display multidrug-resistant Mtb (MDR-TB) results in the drug sensitivity test, which might change a treatment regimen and increase disease burden. Following, we conducted a small-scale surveillance which showed that the isolation rate of S. maltophilia in TB patients was 6.74%, but these patients had no special characteristics and the presence of S. maltophilia was hidden. The effect of S.

maltophilus on TB and its mechanism are unclear and require more attention. IMPORTANCE China is a high-burden country for tuberculosis (TB), multidrug-resistant/rifampicin-resistant tuberculosis (MDR/RR-TB), and HIV-associated TB. Increasing the positive rate of culture and the accuracy of antibiotic susceptibility testing (AST) are important for diagnosis, treatment, and control of TB. In our study, we found that the isolation rate of Stenotrophomonas maltophilia in TB patients was not neglectable and that this bacterium affects the isolation and AST results of TB. Due to a lack of relevant research, the impact of S. maltophilia on the course and outcome of TB is unclear. However, the characteristics of S. maltophilia that increase disease mortality require attention. Therefore, in the clinical testing of TB, in addition to mycobacteria, it is recommended to increase the detection of co-infected bacteria and improve the awareness of TB clinicians of these bacteria.

DOI: 10.1128/spectrum.00944-23
PMID: 37306591

15. **Clinical and imaging features of drug-susceptible and multidrug-resistant TB in Korean adults.**

Int J Tuberc Lung Dis. 2023 Jun 1;27(6):487-489. doi: 10.5588/ijtld.23.0017.

Kim SH(1), Yoo JY(2), Cho HS(2), Kim SR(3), Cho JY(1), Youk S(4), Kim EG(5), Shin YM(1), Choe KH(1), Lee KM(1), Lee H(6), Yang B(1).

DOI: 10.5588/ijtld.23.0017
PMCID: PMC10237266
PMID: 37231602 [Indexed for MEDLINE]

Conflict of interest statement: Conflicts of interest: none declared.

16. **QcrB inhibition as a potential approach for the treatment of tuberculosis: A review of recent developments, patents, and future directions.**

J Infect Public Health. 2023 Jun;16(6):928-937. doi: 10.1016/j.jiph.2023.04.011. Epub 2023 Apr 13.

Imran M(1), Abida(2), Alotaibi NM(3), Thabet HK(4), Alruwaili JA(5), Asdaq SMB(6), Eltaib L(7), Alshehri A(8), Alsaiari AA(9), Almehmadi M(9), Alshammari ABH(10), Alshammari AM(10).

The unmet medical need for drug-resistant tuberculosis (DRTB) is a significant concern. Accordingly, identifying new drug targets for tuberculosis (TB) treatment and developing new therapies based on these drug targets is one of the strategies to tackle DRTB. QcrB is an innovative drug target to create treatments for DRTB. This article highlights QcrB inhibitors and their therapeutic compositions for treating TB. The literature for this article was gathered from PubMed and free patent databases utilizing different keywords related to QcrB inhibitor-based inventions. The data was collected from the conceptualization of telacebec (2010) QcrB to December 2022. A little interesting and encouraging research has been performed on QcrB inhibitors. Telacebec and TB47 are established QcrB inhibitors in the clinical trial. The inventive QcrB inhibitor-based drug combinations can potentially handle DRTB and reduce the TB therapy duration. The authors anticipate great opportunities in fostering QcrB inhibitor-based patentable pharmaceutical inventions against TB. Drug repurposing can be a promising strategy to get safe and effective QcrB inhibitors. However, developing drug resistance, drug tolerance, and selectivity of QcrB inhibitors for Mtb will be the main challenges in developing effective QcrB inhibitors. In conclusion, QcrB is a promising drug target for developing effective treatments for active, latent, and drug-resistant TB. Many inventive and patentable combinations and compositions of QcrB inhibitors with other anti-TB drugs are anticipated as future treatments for TB.

DOI: 10.1016/j.jiph.2023.04.011
PMID: 37086552 [Indexed for MEDLINE]

Conflict of interest statement: Declaration of Competing Interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

17. **Analysis of Dynamic Efficacy Endpoints of the Nix-TB Trial.**

Clin Infect Dis. 2023 Jun 8;76(11):1903-1910. doi: 10.1093/cid/ciad051.

Solans BP(1)(2), Imperial MZ(1)(2), Olugbosi M(3), Savic RM(1)(2).

BACKGROUND: Safer, better, and shorter treatments for multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis (TB) are an urgent global health need. The phase 3 clinical trial Nix-TB (NCT02333799) tested a 6-month treatment of MDR and XDR-TB consisting of high-dose linezolid, bedaquiline, and pretomanid (BPaL). In this study, we investigate the relationship between the

pharmacokinetic characteristics of the drugs, patient characteristics and efficacy endpoints from Nix-TB.
METHODS: Pharmacokinetic data were collected at weeks 2, 8, and 16. Efficacy endpoints including treatment outcomes, time to stable culture conversion, and longitudinal time to positivity in the mycobacterial growth indicator tube assay were each characterized using nonlinear mixed-effects modeling. Relationships between patient, treatment pharmacokinetics, and disease characteristics and efficacy endpoints were evaluated.
RESULTS: Data from 93 (85% of the total) participants were analyzed. Higher body mass index was associated with a lower incidence of unfavorable treatment outcomes. Median time to stable culture conversion was 3 months in patients with lower baseline burden compared with 4.5 months in patients with high baseline burden. Participants with minimal disease had steeper time to positivity trajectories compared with participants with high-risk phenotypes. No relationship between any drugs' pharmacokinetics (drug concentration or exposure metrics) and any efficacy outcomes was observed.
CONCLUSIONS: We have successfully described efficacy endpoints of a BPaL regimen from the Nix-TB trial. Participants with high-risk phenotypes significantly delayed time to culture conversion and bacterial clearance. The lack of a relationship between pharmacokinetic exposures and pharmacodynamic biomarkers opens the possibility to use lower, safer doses, particularly for toxicity-prone linezolid.
CLINICAL TRIALS REGISTRATION: NCT02333799.

DOI: 10.1093/cid/ciad051
PMCID: PMC10249992
PMID: 36804834 [Indexed for MEDLINE]

Conflict of interest statement: Potential conflicts of interest. The authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

18. **Public health benefits of shifting from hospital-focused to ambulatory TB care in Eastern Europe: Optimising TB investments in Belarus, the Republic of Moldova, and Romania.**

PLOS Glob Public Health. 2023 Jun 21;3(6):e0001025. doi: 10.1371/journal.pgph.0001025. eCollection 2023.

Kelly SL(1), Jaoude GJA(2), Palmer T(2), Skordis J(2), Haghparast-Bidgoli H(2),

Goscé L(2)(3), Jarvis SJ(1), Kedziora DJ(4), Abeysuriya R(1), Benedikt C(5), Fraser-Hurt N(5), Shubber Z(5), Cheikh N(5), Bivol S(6), Roberts A(1), Wilson DP(1), Martin-Hughes R(1).

High rates of drug-resistant tuberculosis (DR-TB) continue to threaten public health, especially in Eastern Europe. Costs for treating DR-TB are substantially higher than treating drug-susceptible TB, and higher yet if DR-TB services are delivered in hospital. The WHO recommends that multidrug-resistant (MDR) TB be treated using mainly ambulatory care, shown to have non-inferior health outcomes, however, there has been a delay to transition away from hospital-focused MDR-TB care in certain Eastern European countries. Allocative efficiency analyses were conducted for three countries in Eastern Europe, Belarus, the Republic of Moldova, and Romania, to minimise a combination of TB incidence, prevalence, and mortality by 2035. A primary focus of these studies was to determine the health benefits and financial savings that could be realised if DR-TB service delivery shifted from hospital-focused to ambulatory care. Here we provide a comprehensive assessment of findings from these studies to demonstrate the collective benefit of transitioning from hospital-focused to ambulatory TB care, and to address common regional considerations. We highlight that transitioning from hospital-focused to ambulatory TB care could reduce treatment costs by 20% in Romania, 24% in Moldova, and by as much as 40% in Belarus or almost 35 million US dollars across these three countries by 2035 without affecting quality of care. Improved TB outcomes could be achieved, however, without additional spending by reinvesting these savings in higher-impact TB diagnosis and more efficacious DR-TB treatment regimens. We found commonalities in the large portion of TB cases treated in hospital across these three regional countries, and similar obstacles to transitioning to ambulatory care. National governments in the Eastern European region should examine barriers delaying adoption of ambulatory DR-TB care and consider lost opportunities caused by delays in switching to more efficient treatment modes.

DOI: 10.1371/journal.pgph.0001025
PMCID: PMC10284374
PMID: 37343015

Conflict of interest statement: The authors have declared that no competing interests exist.

19. **Insight into Population Structure and Drug Resistance of Pediatric Tuberculosis Strains from China and Russia Gained through Whole-Genome Sequencing.**

Int J Mol Sci. 2023 Jun 18;24(12):10302. doi: 10.3390/ijms241210302.

Zhdanova S(1), Jiao WW(2), Sinkov V(1), Khromova P(1), Solovieva N(3), Mushkin A(3), Mokrousov I(4)(5), Belopolskaya O(6), Masharsky A(6), Vyazovaya A(4), Rychkova L(1), Kolesnikova L(1), Zhuravlev V(3), Shen AD(2)(5), Ogarkov O(1).

This study aimed to determine phenotypic and genotypic drug resistance patterns of Mycobacterium tuberculosis strains from children with tuberculosis (TB) in China and Russia, two high-burden countries for multi/extensively-drug resistant (MDR/XDR) TB. Whole-genome sequencing data of M. tuberculosis isolates from China (n = 137) and Russia (n = 60) were analyzed for phylogenetic markers and drug-resistance mutations, followed by comparison with phenotypic susceptibility data. The Beijing genotype was detected in 126 Chinese and 50 Russian isolates. The Euro-American lineage was detected in 10 Russian and 11 Chinese isolates. In the Russian collection, the Beijing genotype and Beijing B0/W148-cluster were dominated by MDR strains (68% and 94%, respectively). Ninety percent of B0/W148 strains were phenotypically pre-XDR. In the Chinese collection, neither of the Beijing sublineages was associated with MDR/pre-XDR status. MDR was mostly caused by low fitness cost mutations (rpoB S450L, katG S315T, rpsL K43R). Chinese rifampicin-resistant strains demonstrated a higher diversity of resistance mutations than Russian isolates (p = 0.003). The rifampicin and isoniazid resistance compensatory mutations were detected in some MDR strains, but they were not widespread. The molecular mechanisms of M. tuberculosis adaptation to anti-TB treatment are not unique to the pediatric strains, but they reflect the general situation with TB in Russia and China.

DOI: 10.3390/ijms241210302
PMCID: PMC10299545
PMID: 37373451 [Indexed for MEDLINE]

Conflict of interest statement: The authors declare no conflict of interest.

20. **New Horizons in the Diagnosis of Tuberculosis of the Spine: The Role of Whole Genome Sequencing.**

Asian Spine J. 2023 Jun;17(3):511-517. doi: 10.31616/asj.2022.0247. Epub 2023 May 17.

Ngcelwane M(1), Omar SV(2), Said M(3), Bida M(4).

STUDY DESIGN: Prospective study.
PURPOSE: To evaluate the utility of whole genome sequencing (WGS) in drug resistance testing, lineage of the organisms, and organism- related factors responsible for bacilli settling in the spine.
OVERVIEW OF LITERATURE: The workstream for the diagnosis of tuberculosis (TB) involves isolation and culture of the organism and drug resistance testing using phenotypic methods. Xpert MTB/RIF Ultra is a genetic-based method that detects for Mycobacterium tuberculosis DNA in the rpoB gene. Meanwhile, WGS is a newer genetic-based method that assesses the whole genome of the bacterium. Very few studies have reported the use of WGS for extrapulmonary TB. Herein, we used WGS to diagnose spinal TB.
METHODS: Tissues from 61 patients undergoing surgery for spinal TB underwent histologic examination, Xpert MTB/RIF Ultra, and culture and sensitivity testing. DNA from the cultured bacteria was sent for WGS. The test bacterial genome was compared to a reference strain of pulmonary TB.
RESULTS: Acid-fast bacilli were observed in 9/58 specimens. Meanwhile, histology confirmed TB in all the patients. Bacilli were cultured in 28 patients (48.3%), and the average time to culture was 18.7 days. Xpert MTB/RIF Ultra was positive in 47 patients (85%). WGS was performed in 23 specimens. Overall, 45% of the strains belonged to lineage 2 (East Asian). There was one case of multidrug-resistant TB and two cases of non-tuberculous mycobacteria on WGS. We could not confirm any genomic difference between pulmonary and spinal TB strains.
CONCLUSIONS: Xpert MTB/RIF Ultra of tissues or pus is the investigation of choice when diagnosing spinal TB. Meanwhile, WGS can diagnose multidrug-resistant TB and non-tuberculous mycobacteria more accurately. No mutations were identified in spinal and pulmonary TB bacteria.

DOI: 10.31616/asj.2022.0247
PMCID: PMC10300884
PMID: 37194130

Conflict of interest statement: Conflict of Interest No potential conflict of interest relevant to this article was reported.

21. **Effects of Bedaquiline Combined with Fluoroquinolone and/or Clofazimine on QT Interval in Patients with Multidrug-Resistant Tuberculosis: a Retrospective Study.**

Microbiol Spectr. 2023 Jun 13:e0104823. doi: 10.1128/spectrum.01048-23. Online ahead of print.

Li R(1), Ma JB(1), Yang H(1), Yang H(2), Yang XJ(1), Wu YQ(1), Ren F(1).

With the application of bedaquiline (Bdq), the success rate of multidrug-resistant tuberculosis (MDR-TB) treatment has been significantly improved; however, the cardiac safety of the patients during treatment cannot be ignored. Hence, this study compared the effects of bedaquiline alone and bedaquiline combined with fluoroquinolones (FQs) and/or clofazimine (CFZ) on the QT interval. This single-center retrospective cohort study analyzed the clinical data of MDR-TB patients treated with bedaquiline for 24 weeks from January 2020 to May 2021 in Xi'an Chest Hospital and compared the changes in QTcF between the two groups. Eighty-five patients were included in the study and grouped by types of anti-TB drugs affecting the QT interval they used. Group A included bedaquiline (n = 33), and group B included bedaquiline in combination with fluoroquinolones and/or clofazimine (n = 52). Out of patients with available corrected QT interval by Fridericia's formula (QTcF) data, 2.4% (2/85) experienced a postbaseline QTcF of ≥500 ms, and 24.7% (21/85) had at least one change of QTcF of ≥60 ms from baseline. In group A, 9.1% (3/33) had at least one ΔQTcF of >60 ms, as did 34.6% (18/52) of group B. Multivariate Cox regression analysis showed that the adjusted risk of QT prolongation was 4.82 times higher in group B (95% confidence interval [CI], 1.406 to 16.488). Bedaquiline combined with other anti-TB drugs affecting QT interval significantly increased the incidence of grade 3 or 4 QT prolongation; however, no serious ventricular arrhythmia and permanent drug withdrawal occurred. The use of bedaquiline combined with fluoroquinolone and/or clofazimine is an independent risk factor affecting QT interval. IMPORTANCE Tuberculosis (TB) is a chronic infectious disease caused by Mycobacterium tuberculosis. The emergence of MDR-TB is caused by an organism that is resistant to at least isoniazid and rifampin and is currently considered the major challenge for the global control of TB. Bedaquiline is the first new TB drug in 50 years with a unique mechanism of action, strong anti-M. tuberculosis activity. Yet unexplained excess deaths in the bedaquiline arms have been found in some phase II clinical trials; thus, the FDA has issued a "boxed warning." However, the cardiac safety of the patients during treatment cannot be ignored. Accordingly, further investigations are needed to establish whether bedaquiline combined with clofazimine, fluoroquinolones, or anti-TB drugs affecting the QT interval in a long-course or short-course treatment increases the risk of QT prolongation.

DOI: 10.1128/spectrum.01048-23
PMID: 37310268

22. **Studying the efficacy of isolation as a control strategy and elimination of tuberculosis in India: A mathematical model.**

Infect Dis Model. 2023 Apr 6;8(2):458-470. doi: 10.1016/j.idm.2023.03.005. eCollection 2023 Jun.

Bhadauria AS(1), Dhungana HN(2), Verma V(3), Woodcock S(2), Rai T(2).

India has the highest burden of both tuberculosis (TB) and multidrug-resistant TB (MDR-TB) based on the WHO Global TB Report 2019. Although the available data suggest that the total TB incidence has declined, the absolute number of new cases is still increasing. The number of reported TB cases in India in 2018 was 2.2 million, which was 1.5 million in 2009. About 47% increment in TB case notification in India within a decade shows a persistent public health problem. India contributes about 22% of the World's TB burden. Indian National Strategic Plan 2017-2025, sets out the government plans to eliminate TB by 2025. However, the milestone seems unrealistic to achieve the TB eradication goal by 2025. We developed a five-dimensional mathematical model to understand the TB dynamics in India and investigate the possibility of the earliest TB eradication time frame. The model stratifies the entire TB class into three different classes as drug-sensitive (DS), MDR, and isolated classes. The effective reproduction number, equilibrium points, and stability analysis of the model were carried out. This model predicts the total estimated cases of DS-TB and MDR-TB from 2018 to 2035 through numerical simulation and suggests that TB may be eliminated by 2035 in India if the treatment success rate could be achieved to 95%, by contact tracing and isolating at least 50% of MDR-TB.

DOI: 10.1016/j.idm.2023.03.005
PMCID: PMC10206434
PMID: 37234098

Conflict of interest statement: None.

23. **Adaptive evaluation of mHealth and conventional adherence support interventions to optimize outcomes with new treatment regimens for drug-resistant tuberculosis and HIV in South Africa (ADAP-TIV): Study protocol for an adaptive randomized controlled trial.**

Res Sq. 2023 Jun 9:rs.3.rs-2841179. doi: 10.21203/rs.3.rs-2841179/v1. Preprint.

Ross JE, Perumal R, Wolf A, Zulu M, Guzman K, Seepamore B, Reis K, Nyilana H, Hlathi S, Narasimmulu R, Cheung YKK, Amico KR, Friedland G, Daftary A, Zelnick J, Naidoo K, O'Donnell MR.

Background Highly effective, short course, bedaquiline-containing treatment regimens for multidrug-resistant tuberculosis (MDR-TB) and integrase strand

transfer inhibitor (INSTI)-containing fixed dose combination antiretroviral therapy (ART) have radically transformed treatment for MDR-TB and HIV. However, without advances in adherence support, we may not realize the full potential of these therapeutics. The primary objective of this study is to compare the effect of adherence support interventions on clinical and biological endpoints using an adaptive randomized platform. Methods This is a prospective, adaptive, randomized controlled trial comparing the effectiveness of four adherence support strategies on a composite clinical outcome in adults with MDR-TB and HIV initiating bedaquiline-containing MDR-TB treatment regimens and receiving ART in KwaZulu-Natal, South Africa. Trial arms include 1) enhanced standard of care; 2) psychosocial support; 3) mHealth using cellular-enabled electronic dose monitoring; 4) combined mHealth and psychosocial support. The level of support will be titrated using a differentiated service delivery (DSD)-informed assessment of treatment support needs. The composite primary outcome will be include survival, negative TB culture, retention in care and undetectable HIV viral load at month 12. Secondary outcomes will include individual components of the primary outcome and quantitative evaluation of adherence on TB and HIV treatment outcomes. Discussion This trial will evaluate the contribution of different modes of adherence support on MDR-TB and HIV outcomes with WHO recommended all-oral MDR-TB regimens and ART in a high-burden operational setting. We will also assess the utility of a DSD framework to pragmatically adjust levels of MDR-TB and HIV treatment support. Trial Registration ClinicalTrials.gov: NCT05633056 | December 1, 2022 Funded by The National Institutes of Health (NIH). Grant # R01 AI167798-01A1 (MO).

DOI: 10.21203/rs.3.rs-2841179/v1
PMCID: PMC10274958
PMID: 37333087

Conflict of interest statement: Competing interests The authors declare that they have no competing interests.

24. **Time to Sputum Culture Conversion and Its Predictors Among Multidrug Resistant Tuberculosis Patients in Tigray, Northern Ethiopia: Retrospective Cohort Study.**

Infect Drug Resist. 2023 Jun 9;16:3671-3681. doi: 10.2147/IDR.S413495. eCollection 2023.

Weldemhret L(1), Atsbaha AH(1), Bekuretsion H(1), Desta A(1), Legesse L(1), Kahsay AG(2), Hagos D(2).

BACKGROUND: Sputum culture conversion status is a cardinal index of treatment response and patient outcome for MDR TB patients on longer anti-TB drugs. But,

there is limited information on time to sputum culture conversion of MDR TB patients on a longer anti-TB treatment regimen. Therefore, this study aimed to evaluate time to sputum culture conversion and its predictors among MDR TB patients in Tigray, Northern Ethiopia.
METHODS: A retrospective cohort study was conducted from January 2017 through September 2020 among MDR TB patients in Tigray, Northern Ethiopia. Demographic and clinical characteristics including bacteriological data were extracted from the TB registration book and electronic database in Tigray Health Research Institute. Statistical analysis was performed using SPSS version 25. The time to initial sputum culture conversion was analyzed using the Kaplan-Meier method. Bivariate and multivariate Cox proportional hazards regression analyses were used to identify predictors for culture conversions. P <0.05 was considered statistically significant.
RESULTS: A total of 294 eligible study participants with a median age of 30 years (IQR: 22.75-40) were included. The participants were followed for a total of 1066.7 person months. Sputum culture conversion was achieved in 269 (91%) of the study participants. The median time of sputum culture conversion was 64 days (IQR: 49-86). In our multivariate model, HIV-positive (aHR=1.529, 95% CI: 1.096-2.132, P=0.012), patients new to anti-TB treatment (aHR=2.093, 95% CI: 1.100-3.982, P=0.024) and baseline AFB smear grading of +1 (aHR=1.982, 95% CI: 1.428-2.750, P=0.001) significantly affected time to initial sputum culture conversion.
CONCLUSION: The median time of culture conversion was 64 days. Moreover, the majority of the study participants achieved culture conversion within the first six months of treatment commencement, which supports predefined standard treatment durations.

DOI: 10.2147/IDR.S413495
PMCID: PMC10263018
PMID: 37324659

Conflict of interest statement: The authors declare that there is no competing interest.

25. **Costs of services and funding gap of the Bangladesh National Tuberculosis Control Programme 2016-2022: An ingredient based approach.**

PLoS One. 2023 Jun 2;18(6):e0286560. doi: 10.1371/journal.pone.0286560. eCollection 2023.

Hasan MZ(1)(2), Ahmed S(3), Islam Z(1), Dorin F(1), Rabbani MG(1), Mehdi GG(1),

Ahmed MW(1), Tahsina T(4), Mahmood SS(1), Islam Z(1).

BACKGROUND: Bangladesh National Tuberculosis (TB) Control Programme (NTP) has deployed improved diagnostic technologies which may drive up the programme costs. We aimed to estimate the supply-side costs associated with the delivery of the NTP and the funding gap between the cost of implementation and available funding for the Bangladesh NTP.
METHODS: An ingredient-based costing approach was applied using WHO's OneHealth Tool software. We considered 2016, as the base year and projected cost estimates up to 2022 using information on NTP planned activities. Data were collected through consultative meetings with experts and officials/managers, review of documents and databases, and visits to five purposively selected TB healthcare facilities. The estimated costs were compared with the funds allocated to the NTP between 2018 and 2022 to estimate the funding gap.
FINDINGS: The estimated total cost of NTP was US$ 49.22 million in 2016, which would increase to US$ 146.93 million in 2022. Human resources (41.1%) and medicines and investigations/ supplies (38.0%) were the major two cost components. Unit costs were highest for treating extensively drug-resistant TB at US$ 7,422.4 in 2016. Between 2018-2022, NTP would incur US$ 536.8 million, which is US$ 235.18 million higher than the current allocation for NTP.
CONCLUSION: Our results indicated a funding gap associated with the NTP in each of the years between 2018-2022. Policy planners should advocate for additional funding to ensure smooth delivery of TB services in the upcoming years. The cost estimates of TB services can also be used for planning and budgeting for delivering TB services in similar country contexts.

DOI: 10.1371/journal.pone.0286560
PMCID: PMC10237497
PMID: 37267308 [Indexed for MEDLINE]

Conflict of interest statement: All other authors declare no conflicts of interest.

26. **Ability of the MeltPro MTB/PZA Assay to Detect Susceptibility to Pyrazinamide in Rifampin-Resistant Tuberculosis Patients.**

Microbiol Spectr. 2023 Jun 15;11(3):e0483622. doi: 10.1128/spectrum.04836-22. Epub 2023 May 10.

Li R(#)(1)(2), Li Y(#)(2), Chen X(#)(2), Jia L(#)(3), Yu H(#)(4), Huang Y(5), Wu Q(6), Xiao M(7), Ge S(2), Zhang Y(2), Feng Z(2), Li Q(8), Xu Y(8), Shi W(8), Sun F(2), Zhang W(1)(2)(9)(10)(11).

Prediction of susceptibility to pyrazinamide (PZA) directly from sputum has been challenging. The MeltPro MTB/PZA assay, based on melting curve analysis, can simultaneously detect Mycobacterium tuberculosis and the resistance to PZA from sputum. We aimed to evaluate the MeltPro MTB/PZA assay to predict PZA resistance among rifampin-resistant tuberculosis (RR-TB) patients. We prospectively enrolled RR-TB patients in the registered trials, and their baseline sputum samples were obtained to perform the assay and culture. DNA sequencing of culture isolates was analyzed and used as the reference standard. Sanger sequencing was performed for samples with discrepant results between next-generation sequencing (NGS) and the investigational assay. The main analysis was conducted in the population of patients with interpretable results by both NGS and the assay. A total of 239 patients with RR-TB were screened, and 220 underwent the MeltPro MTB/PZA assay. The assay provided no information for 25 of 220 patients (11.4%). Among the remaining 195 patients, 13 had negative culture or insufficient raw NGS sequencing data, and 15 had indeterminate assay results. A total of 167 patients were included in the main analysis. Against DNA sequencing, the sensitivity, specificity, and negative predictive value of the assay for detecting resistance to PZA were 91.4% (95% confidence interval [CI], 87.1% to 95.6%), 89.9% (95% CI, 85.3% to 94.5%), and 95.2% (95% CI, 91.9% to 98.4%), respectively. In conclusion, the MeltPro MTB/PZA assay is a fast semiautomatic molecular platform to rapidly predict resistance to PZA from sputum and holds promise as a screening tool with satisfactory sensitivity. IMPORTANCE This study evaluated the accuracy of the MeltPro MTB/PZA assay at detecting the presence of PZA resistance through registered clinical trials. Compared to DNA sequencing, the assay had high sensitivity and negative predictive value, suggesting its potential utility as a screening tool in clinical practice. The assay could serve as an ideal primary screening tool in low PZA-resistant M. tuberculosis prevalence settings and could be used as an additional test to identify PZA resistance rapidly and initially in the RR-TB population.

DOI: 10.1128/spectrum.04836-22
PMCID: PMC10269598
PMID: 37162355 [Indexed for MEDLINE]

Conflict of interest statement: The authors declare no conflict of interest.

27. **Whole-Genome Sequence-Based Characterization of Pre-XDR M. tuberculosis Clinical**

Isolates Collected in Kazakhstan.

Diagnostics (Basel). 2023 Jun 8;13(12):2005. doi: 10.3390/diagnostics13122005.

Daniyarov A(1), Akhmetova A(2)(3), Rakhimova S(2), Abilova Z(2), Yerezhepov D(2), Chingissova L(4), Bismilda V(4), Takenov N(4), Akilzhanova A(2), Kairov U(1), Kozhamkulov U(2).

BACKGROUND: Kazakhstan has a high burden of multidrug-resistant tuberculosis in the Central Asian region. This study aimed to perform genomic characterization of Mycobacterium tuberculosis strains obtained from Kazakhstani patients with pre-extensively drug-resistant tuberculosis diagnosed in Kazakhstan.
METHODS: Whole-genome sequencing was performed on 10 pre-extensively drug-resistant M. tuberculosis strains from different regions of Kazakhstan. All strains had high-confidence resistance mutations according to the resistance grading system previously established by the World Health Organization. The genome analysis was performed using TB-Profiler, Mykrobe, CASTB, and ResFinder.
RESULTS: Valuable information for understanding the genetic diversity of tuberculosis in Kazakhstan can also be obtained from whole-genome sequencing. The results from the Phenotypic Drug Susceptibility Testing (DST) of bacterial strains were found to be consistent with the drug resistance information obtained from genomic data that characterized all isolates as pre-XDR. This information can help in developing targeted prevention and control strategies based on the local epidemiology of tuberculosis. Furthermore, the data obtained from whole-genome sequencing can help in tracing the transmission pathways of tuberculosis and facilitating early detection of outbreaks.
CONCLUSIONS: The results from whole-genome sequencing of tuberculosis clinical samples in Kazakhstan provide important insights into the drug resistance patterns and genetic diversity of tuberculosis in the country. These results can contribute to the improvement of tuberculosis control and management programs in Kazakhstan.

DOI: 10.3390/diagnostics13122005
PMCID: PMC10296843
PMID: 37370900

Conflict of interest statement: The authors declare no conflict of interest.

28. **Tuberculosis in times of war and crisis: Epidemiological trends and characteristics of patients born in Ukraine, Germany, 2022.**

Euro Surveill. 2023 Jun;28(24). doi: 10.2807/1560-7917.ES.2023.28.24.2300284.

Hauer B(1), Kröger S(1), Haas W(1), Brodhun B(1).

The Russian invasion of Ukraine in 2022 caused a large migration to other European countries, including Germany. This movement impacted the TB epidemiology, as Ukraine has a higher prevalence of TB and multidrug-resistant TB rates compared to Germany. Our descriptive analysis of TB surveillance data reveals important information to improve TB care in people displaced from Ukraine. We observed an expected increase in the number of TB patients born in Ukraine, which is, however, so far below WHO/Europe estimates.

DOI: 10.2807/1560-7917.ES.2023.28.24.2300284
PMID: 37318760 [Indexed for MEDLINE]

29. **A phase IIb, open-label, randomized controlled dose ranging multi-centre trial to evaluate the safety, tolerability, pharmacokinetics and exposure-response relationship of different doses of delpazolid in combination with bedaquiline delamanid moxifloxacin in adult subjects with newly diagnosed, uncomplicated, smear-positive, drug-sensitive pulmonary tuberculosis.**

Trials. 2023 Jun 6;24(1):382. doi: 10.1186/s13063-023-07354-5.

Dierig A(1)(2), Hoelscher M(1)(2), Schultz S(1)(2), Hoffmann L(1)(2), Jarchow-MacDonald A(1)(2)(3), Svensson EM(4)(5), Te Brake L(4), Aarnoutse R(4), Boeree M(4), McHugh TD(6), Wildner LM(6), Gong X(7), Phillips P(7), Minja LT(8), Ntinginya N(8), Mpagama S(9), Liyoyo A(9), Wallis RS(10), Sebe M(10), Mhimbira FA(11), Mbeya B(11), Rassool M(12), Geiter L(13), Cho YL(13), Heinrich N(14)(15).

BACKGROUND: Linezolid is an effective, but toxic anti-tuberculosis drug that is currently recommended for the treatment of drug-resistant tuberculosis. Improved oxazolidinones should have a better safety profile, while preserving efficacy. Delpazolid is a novel oxazolidinone developed by LegoChem Biosciences Inc. that has been evaluated up to phase 2a clinical trials. Since oxazolidinone toxicity can occur late in treatment, LegoChem Biosciences Inc. and the PanACEA Consortium designed DECODE to be an innovative dose-ranging study with long-term follow-up for determining the exposure-response and exposure-toxicity relationship of delpazolid to support dose selection for later studies. Delpazolid is administered in combination with bedaquiline, delamanid and moxifloxacin.
METHODS: Seventy-five participants with drug-sensitive, pulmonary tuberculosis will receive bedaquiline, delamanid and moxifloxacin, and will be randomized to delpazolid dosages of 0 mg, 400 mg, 800 mg, 1200 mg once daily, or 800 mg twice daily, for 16 weeks. The primary efficacy endpoint will be the rate of decline of bacterial load on treatment, measured by MGIT liquid culture time to detection from weekly sputum cultures. The primary safety endpoint will be the

proportion of oxazolidinone class toxicities; neuropathy, myelosuppression, or tyramine pressor response. Participants who convert to negative liquid media culture by week 8 will stop treatment after the end of their 16-week course and will be observed for relapse until week 52. Participants who do not convert to negative culture will receive continuation phase treatment with rifampicin and isoniazid to complete a six-month treatment course.
DISCUSSION: DECODE is an innovative dose-finding trial, designed to support exposure-response modelling for safe and effective dose selection. The trial design allows assessment of occurrence of late toxicities as observed with linezolid, which is necessary in clinical evaluation of novel oxazolidinones. The primary efficacy endpoint is the change in bacterial load, an endpoint conventionally used in shorter dose-finding trials. Long-term follow-up after shortened treatment is possible through a safety rule excluding slow-and non-responders from potentially poorly performing dosages.
TRIAL REGISTRATION: DECODE was registered in ClinicalTrials.gov before recruitment start on 22 October 2021 (NCT04550832).

DOI: 10.1186/s13063-023-07354-5
PMCID: PMC10243693
PMID: 37280643 [Indexed for MEDLINE]

Conflict of interest statement: All authors except LG and YLC report receiving research funding from LegoChem Biosciences to their institutions. LG receives compensation as a consultant. YLC is an employee of LegoChem Biosciences. Further, the authors would like to report receiving delamanid (Deltyba®) free of charge, from Otsuka Novel Products GmbH.

30. **Inequities between migrants and non-migrants with TB: Surveillance evidence from the Brazilian border State of Roraima.**

One Health. 2022 Dec 9;16:100473. doi: 10.1016/j.onehlt.2022.100473. eCollection 2023 Jun.

de Almeida Soares D(1), Arcêncio RA(2), Fronteira I(1).

INTRODUCTION: Until 2014, there was already a significant burden of TB in Roraima, with this State being among the most affected ones in Brazil. Since 2015, though, there has been a progressive increase in cases of TB in the state of Roraima, with a notorious concentration of cases in Venezuelan migrants. Active international migration in border territories should be seen as a warning signal about the need to strengthen health surveillance and One Health actions

that encompass all components involved in the risk of active transmission of diseases as tuberculosis in these scenarios.
OBJECTIVE: This study aims to analyze and compare migrants and non-migrants notified with TB in the State of Roraima in Brazil and identify inequities in terms of diagnosis, access to treatment and outcome of the disease.
STUDY DESIGN: Quantitative, cross-sectional, descriptive study of all confirmed cases of TB notified in the Information System for Notifiable Diseases (SINAN) between 2009 and 2019.
METHODS: Data were described through counts, frequencies, prevalence ratios and 95% confidence interval. We used Poisson regression with robust variance to adjust for confounders.
RESULTS: 2111 cases of TB were reported in Roraima between 2009 and 2019 and in this study (mean age 38.2 ± 18.5 years). Cases were more frequently males, brownish race, indigenous people, with high school level education. 10.9% (n = 181) of TB cases were migrants, mainly from Venezuela (72.9%). Migrants with TB were more prone to be homeless (PR = 3.7). A higher number of cases of readmission after treatment dropout (3.3%) and AIDS diseases (11.2%) was observed among migrants compared to non-migrants. The proportion of DR-TB was higher among migrants. The percent of cure of TB was lower among migrants and the prevalence of abandonment of treatment, transfers and deaths by other causes was higher compared to non-migrants.
CONCLUSIONS: The results of the study have shown considerable differences in the epidemiological profile of TB between migrants and non-migrants living in the State of Roraima, with a tendency for poorer outcomes in the first ones as well as more concentration of vulnerabilities. These results stress out existing inequities between migrants and non-migrants with TB disease and raise questions on the health care network capacity to address these.

DOI: 10.1016/j.onehlt.2022.100473
PMCID: PMC9791919
PMID: 36578656

Conflict of interest statement: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

31.**Interactome Analysis Identifies MSMEI_3879 as a Substrate of Mycolicibacterium smegmatis ClpC1.**

Microbiol Spectr. 2023 Jun 21:e0454822. doi: 10.1128/spectrum.04548-22. Online ahead of print.

Ogbonna EC(1), Anderson HR(2), Beardslee PC(2), Bheemreddy P(1), Schmitz KR(1)(2).

The prevalence of drug-resistant Mycobacterium tuberculosis infections has prompted extensive efforts to exploit new drug targets in this globally important pathogen. ClpC1, the unfoldase component of the essential ClpC1P1P2 protease, has emerged as one particularly promising antibacterial target. However, efforts to identify and characterize compounds that impinge on ClpC1 activity are constrained by our limited knowledge of Clp protease function and regulation. To expand our understanding of ClpC1 physiology, we employed a coimmunoprecipitation and mass spectrometry workflow to identify proteins that interact with ClpC1 in Mycolicibacterium smegmatis, a surrogate for M. tuberculosis. We identify a diverse panel of interaction partners, many of which coimmunoprecipitate with both the regulatory N-terminal domain and the ATPase core of ClpC1. Notably, our interactome analysis establishes MSMEI_3879, a truncated gene product unique to M. smegmatis, as a novel proteolytic substrate. Degradation of MSMEI_3879 by ClpC1P1P2 in vitro requires exposure of its N-terminal sequence, reinforcing the idea that ClpC1 selectively recognizes disordered motifs on substrates. Fluorescent substrates incorporating MSMEI_3879 may be useful in screening for novel ClpC1-targeting antibiotics to help address the challenge of M. tuberculosis drug resistance. IMPORTANCE Drug-resistant tuberculosis infections are a major challenge to global public health. Much effort has been invested in identifying new drug targets in the causative pathogen, Mycobacterium tuberculosis. One such target is the ClpC1 unfoldase. Compounds have been identified that kill M. tuberculosis by disrupting ClpC1 activity, yet the physiological function of ClpC1 in cells has remained poorly defined. Here, we identify interaction partners of ClpC1 in a model mycobacterium. By building a broader understanding of the role of this prospective drug target, we can more effectively develop compounds that inhibit its essential cellular activities.

DOI: 10.1128/spectrum.04548-22
PMID: 37341639

32. **Baicalein Suppresses NLRP3 and AIM2 Inflammasome-Mediated Pyroptosis in Macrophages Infected by Mycobacterium tuberculosis via Induced Autophagy.**

Microbiol Spectr. 2023 Jun 15;11(3):e0471122. doi: 10.1128/spectrum.04711-22. Epub 2023 May 1.

Ning B(1), Shen J(1), Liu F(1), Zhang H(1), Jiang X(1).

Mycobacterium tuberculosis (Mtb) continues to pose a significant threat to global health because it causes granulomas and systemic inflammatory responses during active tuberculosis (TB). Mtb can induce macrophage pyroptosis, which results in the release of IL-1β and causes tissue damage, thereby promoting its spread. In the absence of anti-TB drugs, host-directed therapy (HDT) has been demonstrated to be an effective strategy against TB. In this study, we used an in vitro Mtb-infected macrophage model to assess the effect of baicalein, derived from Scutellariae radix, on pyroptosis induced in Mtb-infected macrophages. Further, we investigated the molecular mechanisms underlying the actions of baicalein. The results of the study suggest that baicalein inhibits pyroptosis in Mtb-infected macrophages by downregulating the assembly of AIM2 and NLRP3 inflammasome and promoting autophagy. Further research has also shown that the mechanism by which baicalein promotes autophagy may involve the inhibition of the activation of the Akt/mTOR pathway and the inhibition of the AIM2 protein, which affects the levels of CHMP2A protein required to promote autophagy. Thus, our data show that baicalein can inhibit Mtb infection-induced macrophage pyroptosis and has the potential to be a new adjunctive HDT drug. IMPORTANCE Current strategies for treating drug-resistant tuberculosis have limited efficacy and undesirable side effects; hence, research on new treatments, including innovative medications, is required. Host-directed therapy (HDT) has emerged as a viable strategy for modulating host cell responses in order to enhance protective immunity against infections. Baicalein, extracted from Scutellariae radix, was shown to inhibit pyroptosis caused by Mycobacterium tuberculosis-infected macrophages and was associated with autophagy. Our findings reveal that baicalein can be used as an adjunctive treatment for tuberculosis or other inflammatory diseases by regulating immune function and enhancing the antibacterial ability of the host. It also provides a new idea for exploring the anti-inflammatory mechanism of baicalein.

DOI: 10.1128/spectrum.04711-22
PMCID: PMC10269511
PMID: 37125940 [Indexed for MEDLINE]

Conflict of interest statement: The authors declare no conflict of interest.

33. **The Effect of Inoculum Size on Antimicrobial Susceptibility Testing of Mycobacterium tuberculosis.**

Microbiol Spectr. 2023 Jun 15;11(3):e0031923. doi: 10.1128/spectrum.00319-23. Epub 2023 May 22.

Yildirim K(1)(2), Atas C(3), Simsek E(1)(2)(3), Coban AY(1)(2)(3).

Phenotypic drug susceptibility testing (DST) requires a standardized amount of inoculum to produce reproducible susceptibility results. The most critical step in the application of DST in Mycobacterium tuberculosis isolates is the preparation of the bacterial inoculum. In this study, the effect of bacterial inoculum prepared in various McFarland turbidities on primary antituberculosis drug susceptibility of M. tuberculosis strains was investigated. Five standard ATCC strains (ATCC 27294 [H37Rv], ATCC 35822 [izoniazid-resistant], ATCC 35838 [rifampicin-resistant], ATCC 35820 [streptomycin-resistant], ATCC 35837 [ethambutol-resistant]) were tested. Inoculums of McFarland standard of 0.5, 1, 2, 3, and 1:100 dilutions of 1 McFarland standard of each strain were used. The effect of inoculum size on DST results was determined by the proportion method in Lowenstein-Jensen (LJ) medium and nitrate reductase assay (NRA) in the LJ medium. In both test methods, the increase in inoculum size did not affect the DST results of the strains. On the contrary, DST results were obtained more rapidly as a result of the use of dense inoculum. DST results obtained in all McFarland turbidities were found to be 100% compatible with the recommended amount of inoculum, 1:100 dilution of 1 McFarland standard (inoculum size of gold standard method). In conclusion, the use of a high amount of inoculum did not change the drug susceptibility profile of tuberculosis bacilli. Minimizing manipulations during the inoculum preparation phase of susceptibility testing, this outcome will decrease the need for equipment and make the test application easier, particularly in developing countries. IMPORTANCE During DST application, it can be challenging to evenly homogenize TB cell clumps with lipid-rich cell walls. These experiments must be carried out under Biosafety Level-3 (BSL-3) laboratory conditions with personal protective equipment and taking safety precautions because the procedures applied at this stage cause the formation of bacillus-laden aerosols and carry a serious risk of transmission. Considering this situation, this stage is important given that it is not possible to establish a BSL-3 laboratory in poor and developing countries. Reducing the manipulations to be applied during the preparation of bacterial turbidity will minimize the risk of aerosol formation. Perhaps there will be no need to do these steps for susceptibility tests in these countries or even in developed countries.

DOI: 10.1128/spectrum.00319-23
PMCID: PMC10269855
PMID: 37212717 [Indexed for MEDLINE]

Conflict of interest statement: The authors declare no conflict of interest.

34. **Bedaquiline- and clofazimine- selected Mycobacterium tuberculosis mutants: further insights on resistance driven largely by Rv0678.**

Sci Rep. 2023 Jun 27;13(1):10444. doi: 10.1038/s41598-023-36955-y.

Snobre J(#)(1)(2)(3), Villellas MC(#)(4), Coeck N(1), Mulders W(1), Tzfadia O(1), de Jong BC(1), Andries K(4), Rigouts L(5).

Drug-resistant tuberculosis is a serious global health threat. Bedaquiline (BDQ) is a relatively new core drug, targeting the respiratory chain in Mycobacterium tuberculosis (Mtb). While mutations in the BDQ target gene, atpE, are rare in clinical isolates, mutations in the Rv0678 gene, a transcriptional repressor regulating the efflux pump MmpS5-MmpL5, are increasingly observed, and have been linked to worse treatment outcomes. Nevertheless, underlying mechanisms of (cross)-resistance remain incompletely resolved. Our study aims to distinguish resistance associated variants from other polymorphisms, by assessing the in vitro onset of mutations under drug pressure, combined with their impact on minimum inhibitory concentrations (MICs) and on protein stability. For this purpose, isolates were exposed in vitro to sub-lethal concentrations of BDQ or clofazimine (CFZ). Selected colonies had BDQ- and CFZ-MICs determined on 7H10 and 7H11 agar. Sanger sequencing and additional Deeplex Myc-TB and whole genome sequencing (WGS) for a subset of isolates were used to search for mutations in Rv0678, atpE and pepQ. In silico characterization of relevant mutations was performed using computational tools. We found that colonies that grew on BDQ medium had mutations in Rv0678, atpE or pepQ, while CFZ-exposed isolates presented mutations in Rv0678 and pepQ, but none in atpE. Twenty-eight Rv0678 mutations had previously been described among in vitro selected mutants or in patients' isolates, while 85 were new. Mutations were scattered across the Rv0678 gene without apparent hotspot. While most Rv0678 mutations led to an increased BDQ- and/or CFZ-MIC, only a part of them surpassed the critical concentration (69.1% for BDQ and 87.9% for CFZ). Among the mutations leading to elevated MICs for BDQ and CFZ, we report a synonymous Val1Val mutation in the Rv0678 start codon. Finally, in silico characterization of Rv0678 mutations suggests that especially the C46R mutant may render Rv0678 less stable.

DOI: 10.1038/s41598-023-36955-y
PMCID: PMC10300004
PMID: 37369740 [Indexed for MEDLINE]

Conflict of interest statement: J. S. is supported by an FWO PhD fellowship for fundamental research. C. V. and K. A. are employees of Janssen Pharmaceutica. All other authors: none to declare.

35. **Analysis of Xpert MTB/RIF results in retested patients with very low initial**

bacterial loads: A retrospective study in China.

J Infect Public Health. 2023 Jun;16(6):911-916. doi: 10.1016/j.jiph.2023.04.004. Epub 2023 Apr 7.

Wang J(1), Zhang X(2), Huo F(3), Qin L(4), Liu R(1), Shang Y(2), Yao C(2), Ma L(5), Pang Y(6).

BACKGROUND: The Xpert MTB/RIF (Xpert) assay has been widely used to diagnose suspected active tuberculosis (TB) and rifampicin-resistant TB cases. Despite its excellent performance record, false-positive Xpert rifampicin (RIF) resistance results are obtained for specimens with extremely low bacterial loads.
OBJECTIVE: We aimed to study the feasibility of repeat Xpert testing as a strategy for reducing the odds of obtaining false-positive results when testing paucibacillary TB patients.
METHODS: We enrolled previously tested TB patients with very low initial bacterial loads from May 2016 to February 2022 for Xpert retesting. A total of 251 TB patients were retested using the Xpert assay.
RESULTS: RIF resistance was noted in 65 (25.9 %) patients when tested by Xpert at initial diagnosis. Only 107 (42.6 %) of 251 patients tested positive for MTB when retested via Xpert. The majority (98.6 %) of RIF-susceptible cases were still susceptible to RIF when retested. Initial Xpert testing yielded 35 positive results for MTB in the RIF-resistant group, of whom 25 (71.4 %) still exhibited RIF resistance when retested. All culture-positive MTB isolates in the RIF-susceptible group were also RIF-susceptible by phenotypic DST. In the RIF-resistant group, 10 of 14 culture-positive MTB isolates exhibited RIF resistance, of which 4 isolates were deemed RIF-susceptible by phenotypic DST. The proportion of double mutations within the MTB rpoB RRDR sequence, as detected by hybridization of Xpert D and E probes, was significantly higher in the RIF-susceptible group than in the RIF-susceptible group.
CONCLUSIONS: Our results demonstrated that initial RIF-susceptible results were more accurate than RIF-resistant results. Additionally, patients with double mutations that delayed probe D/E hybridization were more likely to have false-positive Xpert results. Our findings emphasize that repeat Xpert MTB/RIF testing is necessary for TB patients with extremely low bacterial loads who are at high risk for RIF-resistant TB.

DOI: 10.1016/j.jiph.2023.04.004
PMID: 37068397 [Indexed for MEDLINE]

Conflict of interest statement: Declaration of Competing Interest The authors

declare that there are no competing financial interests.

36. **Site-directed mutagenesis of Mycobacterium tuberculosis and functional validation to investigate potential bedaquiline resistance-causing mutations.**

Sci Rep. 2023 Jun 6;13(1):9212. doi: 10.1038/s41598-023-35563-0.

Otum CC(1)(2), Rivière E(1), Barnard M(2), Loubser J(2), Williams MJ(2)(3), Streicher EM(1)(2), Van Rie A(1), Warren RM(1)(2), Klopper M(4)(5).

Molecular detection of bedaquiline resistant tuberculosis is challenging as only a small proportion of mutations in candidate bedaquiline resistance genes have been statistically associated with phenotypic resistance. We introduced two mutations, atpE Ile66Val and Rv0678 Thr33Ala, in the Mycobacterium tuberculosis H37Rv reference strain using homologous recombineering or recombination to investigate the phenotypic effect of these mutations. The genotype of the resulting strains was confirmed by Sanger- and whole genome sequencing, and bedaquiline susceptibility was assessed by minimal inhibitory concentration (MIC) assays. The impact of the mutations on protein stability and interactions was predicted using mutation Cutoff Scanning Matrix (mCSM) tools. The atpE Ile66Val mutation did not elevate the MIC above the critical concentration (MIC 0.25-0.5 µg/ml), while the MIC of the Rv0678 Thr33Ala mutant strains (> 1.0 µg/ml) classifies the strain as resistant, confirming clinical findings. In silico analyses confirmed that the atpE Ile66Val mutation minimally disrupts the bedaquiline-ATP synthase interaction, while the Rv0678 Thr33Ala mutation substantially affects the DNA binding affinity of the MmpR transcriptional repressor. Based on a combination of wet-lab and computational methods, our results suggest that the Rv0678 Thr33Ala mutation confers resistance to BDQ, while the atpE Ile66Val mutation does not, but definite proof can only be provided by complementation studies given the presence of secondary mutations.

DOI: 10.1038/s41598-023-35563-0
PMCID: PMC10244393
PMID: 37280265 [Indexed for MEDLINE]

Conflict of interest statement: The authors declare no competing interests.

37. **Microbiological Investigations of Fine Needle Aspirates from Newly Suspected and Previously Treated Tubercular Lymphadenitis Patients.**

Infect Drug Resist. 2023 Jun 1;16:3453-3461. doi: 10.2147/IDR.S407866.
eCollection 2023.

Atnafu A(1), Wassie L(1), Tilahun M(#)(1), Girma S(1), Zenebe Y(2), Beyene MA(1), Alemu A(1), Fisseha E(1), Agze H(1), Desta T(1), Desta K(#)(3), Bobosha K(#)(1).

BACKGROUND: Extrapulmonary tuberculosis (EPTB), particularly tubercular lymphadenitis (TBLN), remains to pose a huge public health problem in Ethiopia. A significant number of TBLN patients who completed a full course anti-TB treatment regimen were reported to have enlarged lymph nodes and other TB-like clinical presentations. This could either be from a paradoxical reaction or microbiological relapse, possibly due to mono/multi-drug resistance.
OBJECTIVE: To investigate the rate of mono and multidrug resistance patterns of Mycobacterium tuberculosis as a cause of the observed treatment failures in clinically diagnosed and anti-TB treatment (newly or previously)-initiated LN patients.
METHODS: A cross-sectional study was conducted on 126 TBLN-suspected and previously treated patients between March and September 2022. Data were analyzed using SPSS (Version 26.0). Descriptive statistics were used to determine the frequency, percentage, sensitivity, specificity, and positive and negative predictive values. The level of agreement was determined using Cohen's kappa and a Chi-square test was used to measure the association between risk factors and laboratory test outcomes. A P-value <0.05 was considered statistically significant.
RESULTS: Mycobacterium tuberculosis was confirmed in 28.6% (N=36) of the 126 cases using BACTEC MGIT 960 culture detection method. Approximately, 13% (N=16) of the samples were collected from previously treated TBLN patients, of which 5/16 (31.3%) were multi-drug resistant, 7/16 were drug-sensitive and 4/16 were culture negative. To rule out other non-tuberculous agents, all samples were grown on blood and Mycosel agar plates, and no growth was detected.
CONCLUSION: The emergence of drug resistant (DR) TB seems to not just be limited to pulmonary form but also to TBLN. In this study we observed a considerable number of microbiologically confirmed relapses among previously treated cases, possibly indicating the need for confirmation of drug resistance using rapid molecular methods or phenotypical methods during treatment follow up.

DOI: 10.2147/IDR.S407866
PMCID: PMC10241182
PMID: 37283940

Conflict of interest statement: The authors report no conflicts of interest in

this work.

38. **Structure of the d-Cycloserine-Resistant Variant D322N of Alanine Racemase from Mycobacterium tuberculosis.**

ACS Bio Med Chem Au. 2023 Mar 27;3(3):233-239. doi: 10.1021/acsbiomedchemau.2c00074. eCollection 2023 Jun 21.

de Chiara C(1), Prosser GA(1), Ogrodowicz R(2), de Carvalho LPS(1)(3).

Alanine racemase (Alr) is a pyridoxal 5'-phosphate-dependent enzyme that catalyzes the racemization of l-alanine to d-alanine. Alr is one of the two targets of the broad-spectrum antibiotic d-cycloserine (DCS), a structural analogue of d-alanine. Despite being an essential component of regimens used to treat multi- and extensively drug-resistant tuberculosis for almost seven decades, resistance to DCS has not been observed in patients. We previously demonstrated that DCS evades resistance due to an ultralow rate of emergence of mutations. Yet, we identified a single polymorphism (converting Asp322 to Asn) in the alr gene, which arose in 8 out of 11 independent variants identified and that confers resistance. Here, we present the crystal structure of the Alr variant D322N in both the free and DCS-inactivated forms and the characterization of its DCS inactivation mechanism by UV-visible and fluorescence spectroscopy. Comparison of these results with those obtained with wild-type Alr reveals the structural basis of the 240-fold reduced inhibition observed in Alr D322N.

DOI: 10.1021/acsbiomedchemau.2c00074
PMCID: PMC10288493
PMID: 37363078

Conflict of interest statement: The authors declare no competing financial interest.

39. **Treatment Outcomes and Associated Factors among Tuberculosis Patients from Selected Rural Eastern Cape Hospitals: An Ambidirectional Study.**

Trop Med Infect Dis. 2023 Jun 9;8(6):315. doi: 10.3390/tropicalmed8060315.

Faye LM(1), Hosu MC(1), Iruedo J(2), Vasaikar S(1), Nokoyo KA(3), Tsuro U(4), Apalata T(1).

An essential metric for determining the efficacy of tuberculosis (TB) control programs is the evaluation of TB treatment outcomes; this study was conducted to investigate treatment outcomes and associated factors among tuberculosis patients in rural areas of Eastern Cape, South Africa. Assessing treatment outcomes is fundamental to facilitating the End TB Strategy's set target. Clinic records from 457 patients with DR-TB were examined for data collection while 101 patients were followed up prospectively. Data were analyzed using Stata version 17.0. The odds ratio and 95% confidence interval were calculated to check the association between variables. $p \leq 0.05$ was considered statistically significant. Of the 427 participants, 65.8% had successful treatment whilst 34.2% had unsuccessful TB treatment. A total of 61.2% and 39% of the HIV-positive and HIV-negative participants had a successful TB treatment whilst 66% and 34% of both HIV-negative and positive participants had unsuccessful TB treatment. From the 101 patients that were followed up, smokers took longer to have treatment outcomes compared to non-smokers. In the study with HIV/TB co-infection, men predominated. HIV and tuberculosis co-infection made therapy difficult with unfavorable effects on TB management. The treatment success rate (65.8%) was lower than the WHO threshold standard with a high proportion of patients being lost to the follow up. The co-infection of tuberculosis and HIV resulted in undesirable treatment outcomes. Strengthening TB surveillance and control is recommended.

DOI: 10.3390/tropicalmed8060315
PMCID: PMC10300936
PMID: 37368733

Conflict of interest statement: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

40. **Proteomic analysis to identification of hypoxia related markers in spinal tuberculosis: a study based on weighted gene co-expression network analysis and machine learning.**

BMC Med Genomics. 2023 Jun 20;16(1):142. doi: 10.1186/s12920-023-01566-z.

Wu S(1), Liang T(1), Jiang J(1), Zhu J(1), Chen T(1), Zhou C(1), Huang S(1), Yao Y(1), Guo H(1), Ye Z(1), Chen L(1), Chen W(1), Fan B(1), Qin J(1), Liu L(1), Wu S(1), Ma F(1), Zhan X(2), Liu C(3).

OBJECTIVE: This article aims at exploring the role of hypoxia-related genes and

immune cells in spinal tuberculosis and tuberculosis involving other organs.
METHODS: In this study, label-free quantitative proteomics analysis was performed on the intervertebral discs (fibrous cartilaginous tissues) obtained from five spinal tuberculosis (TB) patients. Key proteins associated with hypoxia were identified using molecular complex detection (MCODE), weighted gene co-expression network analysis(WGCNA), least absolute shrinkage and selection operator (LASSO), and support vector machine recursive feature Elimination (SVM-REF) methods, and their diagnostic and predictive values were assessed. Immune cell correlation analysis was then performed using the Single Sample Gene Set Enrichment Analysis (ssGSEA) method. In addition, a pharmaco-transcriptomic analysis was also performed to identify targets for treatment.
RESULTS: The three genes, namely proteasome 20 S subunit beta 9 (PSMB9), signal transducer and activator of transcription 1 (STAT1), and transporter 1 (TAP1), were identified in the present study. The expression of these genes was found to be particularly high in patients with spinal TB and other extrapulmonary TB, as well as in TB and multidrug-resistant TB (p-value < 0.05). They revealed high diagnostic and predictive values and were closely related to the expression of multiple immune cells (p-value < 0.05). It was inferred that the expression of PSMB9, STAT 1, and TAP1 could be regulated by different medicinal chemicals.
CONCLUSION: PSMB9, STAT1, and TAP1, might play a key role in the pathogenesis of TB, including spinal TB, and the protein product of the genes can be served as diagnostic markers and potential therapeutic target for TB.

DOI: 10.1186/s12920-023-01566-z
PMCID: PMC10280914
PMID: 37340462 [Indexed for MEDLINE]

Conflict of interest statement: The authors declare no competing interests.

41. **Competing risk models to evaluate the factors for time to loss to follow-up among tuberculosis patients at Ambo General Hospital.**

Arch Public Health. 2023 Jun 25;81(1):117. doi: 10.1186/s13690-023-01130-2.

Fufa DB(#)(1)(2), Diriba TA(#)(3), Dame KT(1), Debusho LK(4).

BACKGROUND: A major challenge for most tuberculosis programs is the inability of tuberculosis patients to complete treatment for one reason or another. Failure to complete the treatment contributes to the emergence of multidrug-resistant TB. This study aimed to evaluate the risk factors for time to loss to follow-up treatment by considering death as a competing risk event among tuberculosis

patients admitted to directly observed treatment short course at Ambo General Hospital, Ambo, Ethiopia.
METHODS: Data collected from 457 tuberculosis patients from January 2018 to January 2022 were used for the analysis. The cause-specific hazard and sub-distribution hazard models for competing risks were used to model the outcome of interest and to identify the prognostic factors associated to treatment loss to follow-up. Loss to follow-up was used as an outcome measure and death as a competing event.
RESULTS: Of the 457 tuberculosis patients enrolled, 54 (11.8%) were loss to follow-up their treatment and 33 (7.2%) died during the follow up period. The median time of loss to follow-up starting from the date of treatment initiation was 4.2 months. The cause-specific hazard and sub-distribution hazard models revealed that sex, place of residence, HIV status, contact history, age and baseline weights of patients were significant risk factors associated with time to loss to follow-up treatment. The findings showed that the estimates of the covariates effects were different for the cause specific and sub-distribution hazard models. The maximum relative difference observed for the covariate between the cause specific and sub-distribution hazard ratios was 12.2%.
CONCLUSIONS: Patients who were male, rural residents, HIV positive, and aged 41 years or older were at higher risk of loss to follow-up their treatment. This underlines the need that tuberculosis patients, especially those in risk categories, be made aware of the length of the directly observed treatment short course and the effects of discontinuing treatment.

DOI: 10.1186/s13690-023-01130-2
PMCID: PMC10290796
PMID: 37357257

Conflict of interest statement: The authors declare no competing interests.

42. **Better understanding extrapulmonary tuberculosis: A scoping review of public health impact in Pakistan, Afghanistan, India, and Bangladesh.**

Health Sci Rep. 2023 Jun 22;6(6):e1357. doi: 10.1002/hsr2.1357. eCollection 2023 Jun.

Jawed A(1), Tharwani ZH(1), Siddiqui A(2), Masood W(1), Qamar K(1), Islam Z(1), Jawed A(3), Shah M(4), Adnan A(2), Essar MY(5), Rackimuthu S(6), Head MG(7).

BACKGROUND AND AIMS: South Asian countries, including Pakistan, Afghanistan, India, and Bangladesh, have a high prevalence of pulmonary and extra-pulmonary

tuberculosis (EPTB). This prevalence is influenced by various risk factors such as ethnicity, nutrition, socioeconomic disparities, high out-of-pocket healthcare expenses, and specific Mycobacterium Tuberculosis (TB) lineages. The COVID-19 pandemic has likely hindered access to healthcare and led to under-reporting of EPTB cases nationally and internationally. This rapid review aimed to summarize the literature on the prevalence and disease outcomes of EPTB in the mentioned countries, compare the situations across countries, and provide recommendations for future action.
METHODS: The review utilized PubMed and Google Scholar databases to search for literature on EPTB in South Asian countries. The search string included keywords related to different forms of EPTB and the countries of interest while excluding pulmonary tuberculosis.
RESULTS: The results showed that both TB, including drug-resistant TB, and EPTB are prevalent and burdensome in South Asia. In Pakistan, pleural TB was the most commonly reported form of EPTB, followed by lymph node TB, abdominal TB, osteoarticular TB, Central Nervous System TB, and miliary TB. In India, lymph node TB(LNTB) was more common among EPTB cases. Bangladesh reported a high prevalence of EPTB involving lymph node, pleura, and abdomen, while Afghanistan had a higher prevalence of forms such as LNTB and tuberculous meningitis.
CONCLUSION: In conclusion, the prevalence of EPTB in Pakistan, Afghanistan, India, and Bangladesh is alarmingly high and negatively impacts population health. Effective measures are needed for treatment and management of this condition, along with addressing current and future challenges. Strengthening the evidence base through surveillance and research is crucial to understand the patterns and significant factors related to EPTB, requiring investment in these areas.

DOI: 10.1002/hsr2.1357
PMCID: PMC10287908
PMID: 37359409

Conflict of interest statement: The authors declare no conflict of interest.

PubMed Non-Open Access Articles

43. **Multi-drug resistant and rifampin-resistant tuberculosis in transplant recipients.**

Transpl Infect Dis. 2023 Jun 19:e14088. doi: 10.1111/tid.14088. Online ahead of print.

Abad CLR(1), Razonable RR(2)(3).

BACKGROUND: Management of multidrug-resistant (MDR) and rifampin-resistant (RR) tuberculosis is challenging. Data on transplant recipients is limited. We reviewed published literature to examine treatment choices, outcomes, and adverse effects from MDR-TB/RR-TB treatment in transplant recipients.
METHODS: Multiple databases from inception to 12/2022 were reviewed using the keywords "drug-resistant TB" or "drug-resistant tuberculosis" or "multidrug-resistant TB" or "multidrug-resistant tuberculosis". MDR-TB was defined as resistance to isoniazid (H) and rifampin (R), and RR if resistant to rifampin alone. Cases without patient-level data and reports which did not describe treatment and/or outcomes for MDR-TB were excluded.
RESULTS: A total of 12 patients (10 solid organ transplants and two hematopoietic cell transplants) were included. Of these, 11 were MDR-TB and one was RR-TB. Seven recipients were male. The median age was 41.5 (range 16-60) years. Pre-transplant evaluation for the majority (8/12, 66.7%) did not reveal a prior history of TB or TB treatment, but 9/12 were from TB intermediate or high-burden countries. Seven patients were initially treated with the quadruple first-line anti-TB regimen. Those who had early RR confirmation (5/12) via Xpert MTB/RIF assay were initiated on alternative therapies. Final regimens were individualized based on susceptibility profiles and tolerability. Adverse events were reported in seven recipients, including acute kidney injury (n = 3), cytopenias (n = 3), and jaundice (n = 2). Four recipients died, with two deaths attributable to TB. The remaining eight patients who survived had functioning allografts at the last follow-up.
CONCLUSIONS: MDR-TB treatment in transplant recipients is associated with many complications. Xpert MTB/RIF detected RR early and guided early empiric therapy.

DOI: 10.1111/tid.14088
PMID: 37335213

44. **End tuberculosis by 2035: challenges ahead.**

Future Microbiol. 2023 Jun 7. doi: 10.2217/fmb-2023-0056. Online ahead of print.

Malik AA(1), Sinha S(2), Ehtesham NZ(1), Hasnain SE(1)(3).

DOI: 10.2217/fmb-2023-0056
PMID: 37284776

45. **Diagnosis, treatment and transmission of rifampicin-resistant TB in the Netherlands, 2010-2019.**

Int J Tuberc Lung Dis. 2023 Jun 1;27(6):471-477. doi: 10.5588/ijtld.22.0676.

de Vries G(1), Akkerman O(2), Boeree M(3), van Hest R(4), Kamst M(1), de Lange W(2), Magis-Escurra C(3), Meijer W(5), van Soolingen D(1).

BACKGROUND: New tools for diagnosis and treatment of rifampicin-resistant (RR-) and multidrug-resistant (MDR-) TB have become available in the last decade, including better tests confirming transmission.OBJECTIVE: To analyse transmission risks of MDR/RR-TB in the Netherlands.METHODS: Analysis of national data of patients with MDR/RR-TB notified in 2010-2019, including contact investigation and genotyping data.RESULTS: Patients with MDR/RR-TB (n = 121) were more often female (adjusted odds ratio [aOR] 1.5), foreign-born, previously treated for TB (aOR 5.2) and co-infected with HIV (aOR 2.3) than patients with no MDR/RR-TB. Treatment outcomes were satisfactory, with at least 79% completing treatment. After additional whole-genome sequencing (WGS), five molecular clusters of 16 patients remained. Patients in three clusters could not be epidemiologically linked and were unlikely to have been infected in the Netherlands. The remaining eight (6.6%) patients with MDR/RR-TB belonged to two clusters, and were likely the result of transmission in the Netherlands. Among close contacts of patients with smear-positive pulmonary MDR/RR-TB, 13.4% (n = 38) had TB infection and 1.1% (n = 3) had TB disease. Only six contacts with TB infection were treated with a quinolone-based preventive treatment regimen.CONCLUSION: MDR/RR-TB is effectively controlled in the Netherlands. Preventive treatment options could be considered more frequently in contacts clearly infected by an index patient with MDR-TB.

DOI: 10.5588/ijtld.22.0676
PMID: 37231607 [Indexed for MEDLINE]

46. **Role of non-coding RNAs in tuberculosis and their potential for clinical applications.**

J Appl Microbiol. 2023 Jun 1;134(6):lxad104. doi: 10.1093/jambio/lxad104.

Jumat MI(1), Sarmiento ME(2), Acosta A(2), Chin KL(1).

Tuberculosis (TB), caused by Mycobacterium tuberculosis (Mtb), remains the leading cause of mortality due to infectious diseases, only surpassed in 2020 by COVID-19. Despite the development in diagnostics, therapeutics, and evaluation of new vaccines for TB, this infectious disease remains uncontrollable due to the emergence of multidrug-resistant (MDR) and extremely drug-resistant (XDR) TB, among other factors. The development in transcriptomics (RNomics) has enabled the study of gene expression in TB. It is considered that non-coding

RNAs (ncRNAs) from host [microRNAs (miRNAs)] and Mtb [small RNAs (sRNAs)] are important elements in TB pathogenesis, immune resistance, and susceptibility. Many studies have shown the importance of host miRNAs in regulating immune response against Mtb via in vitro and in vivo mice models. The bacterial sRNAs play a major role in survival, adaptation, and virulence. Here, we review the characterization and function of host and bacteria ncRNAs in TB and their potential use in clinical applications as diagnostic, prognostic, and therapeutic biomarkers.

DOI: 10.1093/jambio/lxad104
PMID: 37197901 [Indexed for MEDLINE]

47. **Synthesis and Anti-Mycobacterium tuberculosis Activity of Imidazo[2,1-b][1,3]oxazine Derivatives against Multidrug-Resistant Strains.**

ChemMedChem. 2023 Jun 15;18(12):e202300015. doi: 10.1002/cmdc.202300015. Epub 2023 Apr 21.

Fernandes GFS(1)(2)(3), Manieri KF(1), Bonjorno AF(1), Campos DL(1), Ribeiro CM(1), Demarqui FM(1), Ruiz DAG(4), Nascimento-Junior NM(4), Denny WA(2), Thompson AM(2), Pavan FR(1), Dos Santos JL(1).

The emergence of multidrug-resistant strains of M. tuberculosis has raised concerns due to the greater difficulties in patient treatment and higher mortality rates. Herein, we revisited the 2-nitro-6,7-dihydro-5H-imidazo[2,1-b][1,3]oxazine scaffold and identified potent new carbamate derivatives having MIC90 values of 0.18-1.63 µM against Mtb H37Rv. Compounds 47-49, 51-53, and 55 exhibited remarkable activity against a panel of clinical isolates, displaying MIC90 values below 0.5 µM. In Mtb-infected macrophages, several compounds demonstrated a 1-log greater reduction in mycobacterial burden than rifampicin and pretomanid. The compounds tested did not exhibit significant cytotoxicity against three cell lines or any toxicity to Galleria mellonella. Furthermore, the imidazo[2,1-b][1,3]oxazine derivatives did not show substantial activity against other bacteria or fungi. Finally, molecular docking studies revealed that the new compounds could interact with the deazaflavin-dependent nitroreductase (Ddn) in a similar manner to pretomanid. Collectively, our findings highlight the chemical universe of imidazo[2,1-b][1,3]oxazines and their promising potential against MDR-TB.

DOI: 10.1002/cmdc.202300015
PMID: 37002895 [Indexed for MEDLINE]

48. **[Treatment of tuberculosis: what is new?].**

Inn Med (Heidelb). 2023 Jul;64(7):701-707. doi: 10.1007/s00108-023-01523-z. Epub 2023 Jun 14.

[Article in German; Abstract available in German from the publisher]

Brehm TT(1)(2), Köhler N(3), Schmiedel S(1)(2), Terhalle E(4), Martensen J(5), Kalsdorf B(3), Kandulla J(6), Heyckendorf J(7), Kuhns M(8), Friesen I(8), Lange C(9)(10)(11)(12).

Never before have so many people around the world been simultaneously affected by tuberculosis. Tuberculosis is the leading cause of death from a bacterial infectious disease worldwide. The World Health Organization's ambitious goal from 2014 of achieving global elimination of tuberculosis does not seem realistic, but on current trends, tuberculosis could be eliminated in the European Union by 2040. Since the beginning of 2022, there have been more innovations for the treatment of tuberculosis than in no other comparable time period before. One month of rifapentine and isoniazid is effective in treating latent tuberculosis infection. However, rifapentine is licensed in the USA but not in the EU and must be imported for individual cases. The duration of the standard treatment for tuberculosis can be shortened to four months but this treatment regimen is also based on rifapentine, in addition to isoniazid, pyrazinamide, and moxifloxacin. The approval of rifapentine in Europe is a much-needed step towards shortening the treatment of tuberculosis. With new drugs an even shorter standard treatment of only 2 months is possible. The treatment of multidrug-resistant/rifampicin-resistant tuberculosis (MDR-/RR-TB) has been shortened to six months, the same length as the standard treatment available in Germany. The combination of bedaquiline, pretomanid, linezolid ± moxifloxacin, cured around 90% of affected patients were cured in studies with a treatment duration of six months. With 19 drugs in clinical trials, the treatment of tuberculosis is expected to continue to improve rapidly in the coming years.

DOI: 10.1007/s00108-023-01523-z
PMID: 37316702 [Indexed for MEDLINE]

49. **Characteristics of plasma exosomes in drug-resistant tuberculosis patients.**

Tuberculosis (Edinb). 2023 Jun 9;141:102359. doi: 10.1016/j.tube.2023.102359. Online ahead of print.

Wu M(1), Yang Q(2), Yang C(2), Han J(2), Liu H(2), Qiao L(1), Duan H(3), Xing L(2), Liu Q(3), Dong L(4), Wang Q(5), Zuo L(6).

BACKGROUND: Increasing prevalence of drug-resistant tuberculosis (DR-TB) poses a major challenge to the early detection and effective control of tuberculosis (TB). Exosomes carrying proteins and nucleic acid mediate intercellular communication between host and pathogen including Mycobacterium tuberculosis. However, molecular events of exosomes indicating the status and development of DR-TB remain unknown. This study determined the proteomics of exosome in DR-TB and explored the potential pathogenesis of DR-TB.
METHODS: Plasma samples were collected from 17 DR-TB patients and 33 non-drug-resistant tuberculosis (NDR-TB) patients using grouped case-control study design. After exosomes of plasma were isolated and confirmed by compositional and morphological measurement for exosomal characteristics, a label-free quantitative proteomics of exosomes was performed and differential protein components were determined via bioinformatics analysis.
RESULTS: Compared with the NDR-TB group, we identified 16 up-regulated proteins and 10 down-regulated proteins in the DR-TB group. The down-regulated proteins were mainly apolipoproteins and mainly enriched in cholesterol metabolism-related pathways. Apolipoproteins family including APOA1, APOB, APOC1 were key proteins in protein-protein interaction network.
CONCLUSION: Differentially expressed proteins in the exosomes may indicate the status of DR-TB from NDR-TB. Apolipoproteins family including APOA1, APOB, APOC1 may be involved in the pathogenesis of DR-TB by regulating cholesterol metabolism via exosomes.

DOI: 10.1016/j.tube.2023.102359
PMID: 37329682

Conflict of interest statement: Declaration of competing interest We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work reported in this paper.

50. **Comparison of the Diagnostic Performance of MeltPro and Next-Generation Sequencing in Determining Fluoroquinolone Resistance in Multidrug-Resistant Tuberculosis Isolates.**

J Mol Diagn. 2023 Jun;25(6):342-351. doi: 10.1016/j.jmoldx.2023.02.003.

Hu Y(1), Chi Y(2), Feng X(1), Yu F(1), Li H(2), Shang Y(2), Pan J(3), Pang Y(4).

This study systematically investigated the performance of MeltPro and next-generation sequencing in the diagnosis of fluoroquinolone (FQ) resistance among multidrug-resistant tuberculosis patients and explored the relationship between nucleotide alteration and the level of phenotypic susceptibility to FQs. From March 2019 to June 2020, a feasibility and validation study with both MeltPro and next-generation sequencing was performed in 126 patients with multidrug-resistant tuberculosis. Using phenotypic drug susceptibility testing as the gold standard, 95.3% (82 of 86) of ofloxacin-resistant isolates were identified correctly by MeltPro. In addition, whole-genome sequencing was able to detect 83 phenotypically ofloxacin-resistant isolates. The isolates with an individual gyrB mutation outside the quinolone resistance-determining region (QRDR) had minimum inhibitory concentrations (MICs) of ≤2 μg/mL. Despite showing low MICs close to the breakpoint for isolates carrying only gyrA_Ala90Val, the combined mutation gyrB_Asp461Asn caused the ofloxacin MIC to be eight higher than that obtained in Mycobacterium tuberculosis (MTB) isolates with the Ala90Val mutation alone (median, 32 μg/mL; P = 0.038). Heteroresistance was observed in 12 of 88 isolates harboring mutations in the QRDRs. In conclusion, our data show that MeltPro and the whole-genome sequencing assay correctly can identify FQ resistance caused by mutations in the gyrA QRDR. The combined gyrB_Asp461Asn mutation may significantly decrease in vitro FQ susceptibility of MTB isolates with low-level-resistance-associated gyrA mutations.

DOI: 10.1016/j.jmoldx.2023.02.003
PMID: 37208048 [Indexed for MEDLINE]

51. **Synthesis, antitubercular profile and molecular docking studies of quinazolinone-based pyridine derivatives against drug-resistant tuberculosis.**

J Biomol Struct Dyn. 2023 Jun 1:1-11. doi: 10.1080/07391102.2023.2217928. Online ahead of print.

Raghu MS(1), Yogesh Kumar K(2), Shamala T(3), Alharti FA(4), Prashanth MK(3), Jeon BH(5).

The promising quinazolinone-based pyridine derivatives (4a-j) were synthesized and subsequently tested for their antimycobacterial activities against the various drug-sensitive and drug-resistant Mycobacterium tuberculosis (Mtb) strains to combat infectious diseases and address growing concerns about the

devastating effects of tuberculosis (TB). Utilizing 1H NMR, 13C NMR, and mass spectra, the structural and molecular confirmation of the synthesized compounds were deciphered. With minimum inhibitory concentration (MIC) values ranging from 0.31 to 19.13 µM, the results showed that compounds 4e and 4f showed promise anti-TB action against both drug-sensitive and drug-resistant TB strains. To study the cytotoxicity of synthesized molecules, normal Vero and mouse macrophage (RAW264.7) cell lines were utilized. Remarkably, it was revealed that at the highest concentration tested, none of the newly synthesized molecules were toxic to the Vero cell line. The binding patterns of the potent compounds 4b, 4e and 4f in the active site of the mycobacterial membrane protein Large 3 (MmpL3) protein are also revealed by molecular docking studies, which has contributed to the development of a structural rationale for Mtb inhibition. The physicochemical characteristics of the compounds were then predicted using theoretical calculations. Overall, the molecular docking results, physiochemical properties, and observed antimycobacterial activity all point to compound 4e with trifluoromethyl and compound 4f with nitro moiety as potential quinazolinone linked pyridine-based MmpL3 inhibitors.Communicated by Ramaswamy H. Sarma.

DOI: 10.1080/07391102.2023.2217928
PMID: 37261798

52. **Costs and import costs of past, present, and future TB drug regimens: a case study for Karakalpakstan, Uzbekistan.**

J Public Health (Oxf). 2023 Jun 14;45(2):481-487. doi: 10.1093/pubmed/fdac124.

Kohler S(1)(2), Sitali N(3), Achar J(4), Paul N(1).

BACKGROUND: Tuberculosis (TB) drugs and their import are costly. We assessed how shorter TB drug regimens, which were non-inferior or superior in recent TB trials, can affect the costs for purchasing and importing TB drugs.
METHODS: We estimated the drug costs and import costs of 39 longer and shorter TB drug regimens using TB drug prices from the Global Drug Facility and import cost estimates for a TB program in Karakalpakstan, Uzbekistan. Drug regimens from recent TB trials were compared with TB drug regimens following present or past World Health Organization recommendations.
RESULTS: We estimated an import cost of $4.19 and a drug cost of $43 per standard 6-month drug-sensitive (DS)-TB regimen. A new 17-week DS-TB regimen from the TBTC Study 31 currently requires more tablets and is more expensive to import ($6.08) and purchase ($233). The TB program can substantially decrease import costs ($2.26-14) and drug costs ($391-2308) per multidrug-resistant (MDR)-TB regimen when using new 6-month or shorter drug regimens from the Nix-TB, NExT, TB PRACTECAL, ZeNix, or BEAT TB trials instead of 9-20-month

regimens with import costs of $9.96-507 and drug costs of $354-15 028. For a commonly used 20-month all-oral, bedaquiline-containing MDR-TB regimen, we estimated costs of $41 for drug import and $1773 for drug purchase.
CONCLUSIONS: The implementation of a new and shorter DS-TB regimen may increase the costs for drug purchase and import. The implementation of new and shorter MDR-TB regimens may decrease the costs for drug purchase and/or drug import.

DOI: 10.1093/pubmed/fdac124
PMID: 36418232 [Indexed for MEDLINE]

53. **Adolescence, multidrug resistant tuberculosis, bedaquine and videotapes.**

An Pediatr (Engl Ed). 2023 Jun 22:S2341-2879(23)00142-4. doi: 10.1016/j.anpede.2023.01.016. Online ahead of print.

Gamell A(1), Latre C(2), López-Ramos MG(2), Noguera-Julian A(3).

DOI: 10.1016/j.anpede.2023.01.016
PMID: 37355456

54. **Updated considerations in the diagnosis and management of tuberculosis infection and disease: integrating the latest evidence-based strategies.**

Expert Rev Anti Infect Ther. 2023 Jun;21(6):595-616. doi: 10.1080/14787210.2023.2207820. Epub 2023 May 8.

Graciaa DS(1), Schechter MC(1), Fetalvero KB(2)(3), Cranmer LM(4)(5)(6), Kempker RR(1), Castro KG(1)(5)(7).

INTRODUCTION: Tuberculosis (TB) is a leading infectious cause of global morbidity and mortality, affecting nearly a quarter of the human population and accounting for over 10 million deaths each year. Over the past several decades, TB incidence and mortality have gradually declined, but 2021 marked a threatening reversal of this trend highlighting the importance of accurate diagnosis and effective treatment of all forms of TB.
AREAS COVERED: This review summarizes advances in TB diagnostics, addresses the treatment of people with TB infection and TB disease including recent evidence for treatment regimens for drug-susceptible and drug-resistant TB, and draws attention to special considerations in children and during pregnancy.
EXPERT OPINION: Improvements in diagnosis and management of TB have expanded the

available options for TB control. Molecular testing has enhanced the detection of TB disease, but better diagnostics are still needed, particularly for certain populations such as children. Novel treatment regimens have shortened treatment and improved outcomes for people with TB. However, important questions remain regarding the optimal management of TB. Work must continue to ensure the potential of the latest developments is realized for all people affected by TB.

DOI: 10.1080/14787210.2023.2207820
PMCID: PMC10227769
PMID: 37128947 [Indexed for MEDLINE]

Conflict of interest statement: Declaration of interest KG Castro has an Intergovernmental Personnel Act (IPA) award to serve as Senior TB Scientific Advisor, Office of Infectious Disease, Bureau for Global Health, U.S. Agency for International Development. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

55. **Current therapeutic delivery approaches using nanocarriers for the treatment of tuberculosis disease.**

Int J Pharm. 2023 Jun 10;640:123018. doi: 10.1016/j.ijpharm.2023.123018. Epub 2023 May 4.

Biswas B(1), Misra TK(2), Ray D(3), Majumder T(3), Bandyopadhyay TK(1), Bhowmick TK(4).

Tuberculosis is a major health issue globally and a leading cause of death due to the infective microorganism Mycobacterium tuberculosis. Treatment of drug resistance tuberculosis requires longer treatment with multiple daily doses of drugs. Unfortunately, these drugs are often associated with poor patient compliance. In this situation, a need has been felt for the less toxic, shorter, and more effective treatment of the infected tuberculosis patients. Current research to develop novel anti-tubercular drugs shows hope for better management of the disease. Research on drug targeting and precise delivery of the old anti-tubercular drugs with the help of nanotechnology is promising for effective treatment. This review has discussed the status currently available treatments for tuberculosis patients infected with Mycobacterium alone or in comorbid conditions like diabetes, HIV and cancer. This review also highlighted the challenges in the current treatment and research on the novel anti-tubercular drugs to prevent multi-drug-resistant tuberculosis. It presents the research highlights on the targeted delivery of anti-tubercular drugs using different nanocarriers for preventing multi-drug resistant tuberculosis. Report has shown

the importance and development of the research on nanocarriers mediated anti-tubercular delivery of the drugs to overcome the current challenges in tuberculosis treatment.

DOI: 10.1016/j.ijpharm.2023.123018
PMID: 37149113 [Indexed for MEDLINE]

Conflict of interest statement: Declaration of Competing Interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

56. **Rapid Detection of Extensive Drug Resistance by Xpert MTB/XDR Optimizes Therapeutic Decision-Making in Rifampin-Resistant Tuberculosis Patients.**

J Clin Microbiol. 2023 Jun 20;61(6):e0183222. doi: 10.1128/jcm.01832-22. Epub 2023 May 30.

Chen X(#)(1), Li R(#)(1), Ge S(#)(1), Li Y(#)(1), Cai C(#)(2), Weng T(1)(3), Zhang Y(1), Jiang J(1), Feng Z(1), Chen Y(4), Zhang Y(5), Ma J(6), Persing DH(6), Chen J(1), Tang YW(6), Sun F(1), Zhang W(1)(7)(8)(9).

The Xpert MTB/XDR assay met the critical need for etiologic diagnosis of tuberculosis and rifampin resistance in previous studies. However, its benefits in tailoring the treatment regimen and improving the outcome for patients with rifampin-resistant tuberculosis (RR-TB) require further investigation. In this study, the Xpert MTB/XDR assay was used to determine the resistance profile of second-line drugs for RR-TB patients in two registered multicenter clinical trials, TB-TRUST (NCT03867136) and TB-TRUST-plus (NCT04717908), with the aim of testing the efficacy of all-oral shorter regimens in RR-TB patients in China. Patients would receive the fluoroquinolone-based all-oral shorter regimen, the injectable-containing regimen, or the bedaquiline-based regimen depending on fluoroquinolone susceptibility by using Xpert MTB/XDR. Among the 497 patients performed with Xpert MTB/XDR, 128 (25.8%) had infections resistant to fluoroquinolones and/or second-line injectable drugs (SLIDs). A total of 371 participants were recruited for the trials, and whole-genome sequencing (WGS) was performed on all corresponding culture-positive baseline strains. Taking the WGS results as the standard, the accuracy of the Xpert MTB/XDR assay in terms of resistance detection was 95.2% to 99.0% for all drugs. A total of 33 cases had inconsistent results, 9 of which were due to resistance heterogeneity. Most of the patients (241/281, 85.8%) had sputum culture conversion at 2 months. In conclusion, the Xpert MTB/XDR assay has the potential to serve as a quick reflex

test in patients with RR-TB, as detected via Xpert MTB/RIF, to provide a reliable drug susceptibility profile of the infecting Mycobacterium tuberculosis strain and to initiate optimized treatment promptly.

DOI: 10.1128/jcm.01832-22
PMCID: PMC10281159
PMID: 37249422 [Indexed for MEDLINE]

Conflict of interest statement: The authors declare no conflict of interest.

57. **[Progress on the safety and efficacy of bedaquiline for the treatment of drug-resistant tuberculosis in special populations].**

Zhonghua Jie He He Hu Xi Za Zhi. 2023 Jun 12;46(6):619-624. doi: 10.3760/cma.j.cn112147-20230103-00003.

Zhang YP(1), Niu YY(2), Tang PJ(2).

Antimicrobial resistance in Mycobacterium tuberculosis is a serious threat to global tuberculosis(TB) control. WHO listed bedaquiline as one of the first-choice drugs for the treatment of MDR/RR-TB in 2018. Bedaquiline is marketed for adult patients with MDR-TB and XDR-TB. However, there are few studies of bedaquiline in adolescents, pregnant women, the elderly, and other special populations with drug-resistant TB. This paper aimed to review the effectiveness and safety of bedaquiline in the treatment of special populations of drug-resistant TB for the clinical use.

DOI: 10.3760/cma.j.cn112147-20230103-00003
PMID: 37278180 [Indexed for MEDLINE]

58. **Co-treatment with Clofazimine and Rapamycin eliminates drug-resistant tuberculosis by inducing polyfunctional central memory T cell responses.**

J Infect Dis. 2023 Jun 8:jiad214. doi: 10.1093/infdis/jiad214. Online ahead of print.

Singh DK(1)(2), Bhaskar A(1), Pahuja I(1), Shaji A(1), Moitra B(2), Shi Y(3), Dwivedi VP(1), Das G(2).

Mycobacterium tuberculosis (M.tb), the causative agent of tuberculosis (TB), is acquiring drug resistance at a faster rate than the discovery of new antibiotics. Therefore, alternate therapies that can limit the drug resistance and disease recurrence are urgently needed. Emerging evidences indicate that combined treatment with antibiotics and an immunomodulator provides superior

treatment efficacy. Clofazimine (CFZ) enhances the generation of T central memory (TCM) cells by blocking the Kv1.3+ potassium channels. Rapamycin (Rapa) facilitates M.tb clearance by inducing autophagy. In this study, we observed that the co-treatment with CFZ and Rapa potently eliminates both multiple and extensively drug-resistant (MDR and XDR) clinical isolates of M.tb in a mouse model by inducing robust T cell memory and polyfunctional TCM responses. Furthermore, co-treatment reduces the expression of latency-associated genes of M.tb in human macrophages. Therefore, CFZ and Rapa co-therapy holds promise for treating patients infected with MDR and XDR strains of M.tb.

DOI: 10.1093/infdis/jiad214
PMID: 37290049

59. **To Test or Not? Xpert MTB/RIF as an Alternative to Smear Microscopy to Guide Line Probe Assay Testing for Drug-Resistant Tuberculosis.**

J Clin Microbiol. 2023 Jun 27:e0001723. doi: 10.1128/jcm.00017-23. Online ahead of print.

Pillay S(1)(2), de Vos M(1), Sohn H(3), Ghebrekristos Y(2), Dolby T(2), Warren RM(1), Theron G(1).

Xpert MTB/RIF (Xpert) revolutionized tuberculosis (TB) diagnosis. Laboratory decision making on whether widely-used reflex drug susceptibility assays (MTBDRplus, first-line resistance; MTBDRsl, second-line) are conducted is based on smear status, with smear-negative specimens often excluded. We performed receiver operator characteristic (ROC) curve analyses using bacterial load information (smear microscopy grade, Xpert-generated semi-quantitation categories and minimum cycle threshold [CTmin] values) from Xpert rifampicin-resistant sputum for the prediction of downstream line probe assay results as "likely non-actionable" (no resistance or susceptible results generated). We evaluated actionable-to-non-actionable result ratios and pay-offs with missed resistance versus LPAs done universally. Smear-negatives were more likely than smear-positive specimens to generate a non-actionable MTBDRplus (23% [133/559] versus 4% [15/381]) or MTBDRsl (39% [220/559] versus 12% [47/381]) result. However, excluding smear-negatives would result in missed rapid diagnoses (e.g., only 49% [264/537] of LPA-diagnosable isoniazid resistance would be detected if smear-negatives were omitted). Testing smear-negatives with a semi-quantitation category ≥ "medium" had a high ratio of actionable-to-non-actionable results (12.8 or a 4-fold improvement versus

testing all using MTBDRplus, 4.5 or 3-fold improvement for MTBDRsl), which would still capture 64% (168/264) and 77% (34/44) of LPA-detectable smear-negative resistance, respectively. Use of CTmins permitted optimization of this ratio with higher specificity for non-actionable results but decreased resistance detected. Xpert quantitative information permits identification of a smear-negative subset in whom the payoffs of the ratio of actionable-to-non-actionable LPA results with missed resistance may prove acceptable to laboratories, depending on context. Our findings permit the rational expansion of direct DST to certain smear-negative sputum specimens.

DOI: 10.1128/jcm.00017-23
PMID: 37367228

60. **Simple and low-cost antibiotic susceptibility testing for Mycobacterium tuberculosis using screen-printed electrodes.**

Biotechnol Appl Biochem. 2023 Jun;70(3):1397-1406. doi: 10.1002/bab.2448. Epub 2023 Feb 24.

Ghorbanpoor H(1)(2)(3)(4)(5), Akcakoca I(6), Norouz Dizaji A(2), Butterworth A(7), Corrigan D(7)(8), Kocagoz T(9)(10), Ebrahimi A(2)(4)(5), Avci H(3)(4)(5)(11), Dogan Guzel F(2).

One quarter of the global population is thought to be latently infected by Mycobacterium tuberculosis (TB) with it estimated that 1 in 10 of those people will go on to develop active disease. Due to the fact that M. tuberculosis (TB) is a disease most often associated with low- and middle-income countries, it is critical that low-cost and easy-to-use technological solutions are developed, which can have a direct impact on diagnosis and prescribing practice for TB. One area where intervention could be particularly useful is antibiotic susceptibility testing (AST). This work presents a low-cost, simple-to-use AST sensor that can detect drug susceptibility on the basis of changing RNA abundance for the typically slow-growing M. tuberculosis (TB) pathogen in 96 h using screen-printed electrodes and standard molecular biology laboratory reactionware. In order to find out the sensitivity of applied sensor platform, a different concentration (108 -103 CFU/mL) of M. tuberculosis was performed, and limit of detection and limit of quantitation were calculated as 103.82 and 1011.59 CFU/mL, respectively. The results display that it was possible to detect TB sequences and distinguish antibiotic-treated cells from untreated cells with a label-free molecular detection. These findings pave the way for the development of a comprehensive, low-cost, and simple-to-use AST system for prescribing in TB and multidrug-resistant tuberculosis.

DOI: 10.1002/bab.2448
PMID: 36738290 [Indexed for MEDLINE

61. **[Analysis of tuberculosis epidemiological characteristics and drug resistance among the floating population in Beijing in 2019].**

Zhonghua Liu Xing Bing Xue Za Zhi. 2023 Jun 10;44(6):949-953. doi: 10.3760/cma.j.cn112338-20221011-00870.

Yang XY(1), Chen SS(1), Yi JL(1), Zhao YF(1), Chen H(1), Dai XW(1), Ding BC(1), Pang MD(1), Li Q(1), Zhao ZY(2), Li CY(1).

Author information:
(1)Tuberculosis Laboratory, Beijing Center for Disease Prevention and Control, Beijing 100013, China.
(2)Fuxing Hospital, Capital Medical University, Beijing 100038, China.

Objective: To analyze the epidemic characteristics and drug resistance of pulmonary tuberculosis among the floating population in Beijing and to provide a scientific basis for formulating strategies for the prevention and control of tuberculosis among the floating population. Methods: Data of tuberculosis patients who were positive for Mycobacterium tuberculosis culture was collected from 16 districts and one municipal institution of tuberculosis control and prevention in Beijing in 2019. The strain samples were tested for drug sensitivity by the proportional method. According to household registration location, patients were divided into the floating population and Beijing registration. SPSS 19.0 software analyzed tuberculosis patients' epidemic characteristics and drug resistance in the floating population. Results: In 2019, there were 1 171 culture-positive tuberculosis patients in Beijing, among the floating population, 593 (50.64%) patients were identified, with a male-to-female sex ratio of 2.2∶1 (409∶184). Compared to patients under household registration as Beijing residents, a higher proportion of young adults aged 20-39 years (65.09%,386/593) were noticed, with 55.65% (330/593) reported from the urban areas and 96.80% (574/593) were reported the first time. The differences were statistically significant (all $P<0.05$). After completing the drug sensitivity test, 37 cases were with multiple drug-resistant tuberculosis, accounting for 6.24% (37/593). The rates of isoniazid resistance (42.11%,8/19) and multidrug resistance (21.05%,4/19) in floating population patients after retreatment were significantly higher than those in newly treated patients (11.67%, 67/574 and 5.75%, 33/574), and the differences were statistically significant (all $P<0.05$). Conclusions: Most patients with tuberculosis in the floating population in Beijing in 2019 were young males aged 20-39 years. The reporting areas were urban areas and the newly treated patients mainly. The

patients with tuberculosis in the re-treated floating population were more likely to suffer from multidrug and drug resistance, which should be taken as the key population for prevention and control.

DOI: 10.3760/cma.j.cn112338-20221011-00870
PMID: 37380418 [Indexed for MEDLINE]

62. **Pharmacogenetics as part of recommended precision medicine for tuberculosis treatment in African populations: Could it be a reality?**

Clin Transl Sci. 2023 Jun 8. doi: 10.1111/cts.13520. Online ahead of print.

Oelofse C(1), Ndong Sima CAA(1), Möller M(1)(2), Uren C(1)(2).

Globally, tuberculosis (TB) is the second most lethal infectious disease. However, in sub-Saharan Africa, TB has the largest disease burden, with drug-resistant TB increasingly becoming a concern. The social and economic impact of TB should not be overlooked, especially in areas where healthcare systems are overburdened, and resources need to be allocated judiciously. The aim of pharmacogenetics (PGx) is to improve therapeutic response and to minimize adverse drug reactions by selecting the most optimal drug and dosage for the individual patient. Implementation of PGx into routine clinical care has been slow, especially in resource-limited settings, because of perceived high costs relative to uncertain benefit. Given the impact of TB on the disease and disability burden in these regions, a better understanding and optimization of TB treatment in understudied African populations is vital. The first weeks of treatment are the most crucial for treatment success, and a point-of-care pre-emptive PGx test could start patients on the most bactericidal and least toxic drug combination. This may potentially reduce the number of patients returning to clinical care and streamline the use of limited resources across the healthcare system. This review explores the status of TB PGx in Africa, the utility of existing TB PGx testing panels, and the economic feasibility in developing a clinically valuable, cost-effective, pre-emptive PGx test to guide optimized, new dosing regimens specifically for African population groups. TB is a disease of poverty, but investment in PGx research in African populations could ensure improved treatments and long-term cost savings.

DOI: 10.1111/cts.13520
PMID: 37291686

63. **Advances of new drugs bedaquiline and delamanid in the treatment of multi-drug**

resistant tuberculosis in children.

Front Cell Infect Microbiol. 2023 Jun 13;13:1183597. doi: 10.3389/fcimb.2023.1183597. eCollection 2023.

Zhu H(1)(2), Zhou X(1), Zhuang Z(1)(2), Li L(1)(3), Bi J(4), Mi K(1)(2).

Tuberculosis (TB) is a major public health problem, with nearly 10 million new cases and millions of deaths each year. Around 10% of these cases are in children, but only a fraction receive proper diagnosis and treatment. The spread of drug-resistant (DR) strain of TB has made it difficult to control, with only 60% of patients responding to treatment. Multi-drug resistant TB (MDR-TB) is often undiagnosed in children due to lack of awareness or under-diagnosis, and the target for children's DR-TB treatment has only been met in 15% of goals. New medications such as bedaquiline and delamanid have been approved for treating DR-TB. However, due to age and weight differences, adults and children require different dosages. The availability of child-friendly formulations is limited by a lack of clinical data in children. This paper reviews the development history of these drugs, their mechanism of action, efficacy, safety potential problems and current use in treating DR-TB in children.

DOI: 10.3389/fcimb.2023.1183597
PMCID: PMC10293792
PMID: 37384221

Conflict of interest statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

64. **Inhaled Adjunct Therapy with Second-Line Drug Candidates for Dose Reduction in Chemotherapeutic Regimens for Multi-drug-Resistant Tuberculosis.**

AAPS PharmSciTech. 2023 Jun 8;24(5):130. doi: 10.1208/s12249-023-02585-w.

Verma S(1)(2), Dal NK(3), Srivastava A(1)(2), Bharti R(1), Siva Reddy DV(1)(2), Sofi HS(1), Roy T(1)(2), Verma K(1)(2), Raman SK(1), Azmi L(1), Ray L(1), Mugale MN(1)(2), Singh AK(4), Singh J(5), Griffiths G(6), Misra A(7)(8).

Chemotherapy of multi-drug-resistant tuberculosis (TB) requires prolonged administration of multiple drugs. We investigated whether pulmonary delivery of minute doses of drugs, along with reduced oral doses of the same agents, would affect preclinical efficacy. We prepared dry powder inhalation (DPI)

formulations comprising sutezolid (SUT), the second-generation pretomanid analog TBA-354 (TBA), or a fluorinated derivative of TBA-354 (32,625) in a matrix of the biodegradable polymer poly(L-lactide). We established formulation characteristics, doses inhaled by healthy mice, and preclinical efficacy in a mouse model of TB. Oral doses of 100 mg/kg/day or DPI doses of 0.25-0.5 mg/kg/day of drugs SUT, TBA-354, or 32,625 administered over 28 days were sub-optimally effective in reducing lung and spleen burden of Mycobacterium tuberculosis (Mtb) in infected mice. The addition of 0.25-0.5 mg/kg/day of SUT, TBA-354, or 32,625 as DPI to oral doses of 50 mg/kg/day was non-inferior in clearing Mtb from the lungs of infected mice. We concluded that adjunct therapy with inhaled second-line agents has the potential to reduce the efficacious oral dose.

DOI: 10.1208/s12249-023-02585-w
PMID: 37291443 [Indexed for MEDLINE]

65. **Long-Term Intake of Linezolid Elevates Drug Exposure and Reduces Drug Clearance and Elimination in Adults With Drug-Resistant Pulmonary Tuberculosis.**

Ther Drug Monit. 2023 Jun 6. doi: 10.1097/FTD.0000000000001111. Online ahead of print.

Jeyakumar SM(1), Bhui NK(2), Singla N(3), Vilvamani S(1), Mariappan MV(1), Padmapriyadarsini C(1), Bhatnagar AK(4), Solanki R(5), Sridhar R(6).

PURPOSE: Pharmacokinetic (PK) studies are critical for dose optimization, and there is a paucity of linezolid (LZD) PK data for prolonged use in drug-resistant tuberculosis (DR-TB). Therefore, the authors evaluated the pharmacokinetics of LZD at two-time intervals in DR-TB during long-term use.
METHODS: PK evaluation of LZD was performed at the end of the 8th and 16th weeks of treatment in a randomly selected subset of adult pre-extensively drug-resistant pulmonary tuberculosis patients (n = 18) from a multicentric interventional study (Building Evidence to Advance Treatment of TB/BEAT study; CTRI/2019/01/017310), wherein a daily dose of 600 mg LZD was used for 24 weeks. Plasma LZD levels were measured using a validated high-pressure liquid chromatography (HPLC) method.
RESULTS: The LZD median plasma Cmax was comparable between the 8th and 16th weeks [18.3 mg/L, interquartile range (IQR: 15.5-20.8 and 18.8 mg/L, IQR: 16.0-22.7, respectively)]. However, the trough concentration increased significantly in the 16th week (3.16 mg/L, IQR: 2.30-4.76), compared with the 8th week (1.98 mg/L, IQR: 0.93-2.75). Furthermore, compared with the 8th week,

in the 16th week, there was a significant increase in drug exposure (AUC0-24 = 184.2 mg*h/L, IQR: 156.4-215.8 versus 233.2 mg*h/L, IQR: 187.9-277.2), which corroborated with a longer elimination half-life (6.94 hours, IQR: 5.55-7.99 versus 8.47 hours, IQR:7.36-11.35) and decreased clearance (2.91 L/h, IQR: 2.45-3.33 versus 2.19 L/h, IQR: 1.49-2.78).
CONCLUSIONS: Long-term daily intake of 600 mg LZD resulted in a significant elevation in trough concentration (>2.0 mg/L) in 83% of the study participants. Furthermore, increased LZD drug exposure may be partly because of decreased clearance and elimination. Overall, the PK data underscore the need for dose adjustment when LZDs are intended for long-term treatment.

DOI: 10.1097/FTD.0000000000001111
PMID: 37296501

Conflict of interest statement: The authors declare no conflict of interest.

66. **[Comprehensive clinical evaluation of bedaquiline in the treatment of multidrug-resistant tuberculosis].**

Zhonghua Jie He He Hu Xi Za Zhi. 2023 Jun 12;46(6):572-579. doi: 10.3760/cma.j.cn112147-20221031-00859.

Geng X(1), Yang Y(1), Wen XT(1), Long HF(2), Li YX(1), Liu YX(2), Mao ZF(1).

Objective: To assess the clinical value of bedaquiline in five dimensions: effectiveness, safety, economics, appropriateness, and social benefits, to provide a reference for medical and health insurance-related decisions. Methods: A total of 792 patients with multidrug-resistant tuberculosis who were hospitalized at Wuhan Pulmonary Hospital, Ganzhou Fifth People's Hospital and Jiangxi Chest Hospital between January 2018 and December 2020 were included in the study. Based on a retrospective survey of case data, and each evaluation dimension of bedaquiline was statistically analyzed by causal analysis or chi-square test, using linezolid as the reference drug. Results: In terms of effectiveness, bedaquiline significantly increased treatment success by 23.9% (95%CI:4.8%-43.0%) and shortened treatment duration by 64 days(95%CI:18-109 days). In terms of safety, the incidence of adverse reactions to bedaquiline and the discontinuation rate of adverse reactions (5.11%,4.55%) were significantly lower than those for linezolid (22.49%,15.24%), with statistically significant differences ($\chi2=27.50$,$P<0.001$;$\chi2=14.09$,$P<0.001$). In terms of economics, patients treated with bedaquiline had a significantly higher anti-TB drug course cost of RMB 48 209.4 Yuan(95%CI: 28 336.0-68 082.8 Yuan). In terms of appropriateness, the proportion of bedaquiline in patients' initial treatment regimens was lower

than that of linezolid (16.7% vs. 86.5%) in the 2020 observation sample, with a statistically significant difference (χ2=238.96,P<0.001). In terms of social benefits, the infection control rate was significantly increased by 27.8% (95%CI:8.2%-47.5%) in patients using bedaquiline. Conclusions: Bedaquiline performed well in terms of efficacy, safety, and social benefits. However, it was less economical and the actual use rate of bedaquiline in clinical practice was lower than that of its counterpart drug, linezolid. Price reductions might be needed to increase the clinical use and performance of bedaquiline in the future.

DOI: 10.3760/cma.j.cn112147-20221031-00859
PMID: 37278171 [Indexed for MEDLINE]

67. **Making the Case for All-Oral, Shorter Regimens for Children with Drug-Resistant Tuberculosis.**

Am J Respir Crit Care Med. 2023 Jun 5. doi: 10.1164/rccm.202304-0670VP. Online ahead of print.

Patankar S(1), Cruz AT(2), Douglas-Jones B(3), Garcia-Prats A(4), Kay A(2), Reuter A(5), Schaaf HS(6), Seddon JA(7)(8), Sharma S(9), Starke J(10), Tommasi M(11), Triasih R(12), Furin JJ(13).

DOI: 10.1164/rccm.202304-0670VP
PMID: 37276531

68. **Quantification of Isoniazid-Heteroresistant Mycobacterium tuberculosis Using Droplet Digital PCR.**

J Clin Microbiol. 2023 Jun 20;61(6):e0188422. doi: 10.1128/jcm.01884-22. Epub 2023 May 17.

Zhang S(#)(1), Chen X(#)(2), Lin Z(#)(2), Tan Y(3), Liang B(1), Pan Y(1), Huang M(2), Su B(3), Hu X(1), Xu Y(1), Li Q(1).

The quantitative detection of drug-resistance mutations in Mycobacterium tuberculosis (MTB) is critical for determining the drug resistance status of a sample. We developed a drop-off droplet digital PCR (ddPCR) assay targeting all major isoniazid (INH)-resistant mutations. The ddPCR assay consisted of three reactions: reaction A detects mutations at katG S315; reaction B detects inhA promoter mutations; and reaction C detects ahpC promoter mutations. All reactions could quantify 1%-50% of mutants in the presence of the wild-type, ranging from 100 to 50,000 copies/reaction. Clinical evaluation with 338 clinical isolates yielded clinical sensitivity of 94.5% (95% confidence interval

[CI] = 89.1%-97.3%) and clinical specificity of 97.6% (95% CI = 94.6%-99.0%) compared with the traditional drug susceptibility testing (DST). Further clinical evaluation using 194 nucleic acid-positive MTB sputum samples revealed clinical sensitivity of 87.8% (95% CI = 75.8%-94.3%) and clinical specificity of 96.5% (95% CI = 92.2%-98.5%) in comparison with DST. All the mutant and heteroresistant samples detected by the ddPCR assay but susceptible by DST were confirmed by combined molecular assays, including Sanger sequencing, mutant-enriched Sanger sequencing and a commercial melting curve analysis-based assay. Finally, the ddPCR assay was used to monitor longitudinally the INH-resistance status and the bacterial load in nine patients undergoing treatment. Overall, the developed ddPCR assay could be an indispensable tool for quantification of INH-resistant mutations in MTB and bacterial loads in patients.

DOI: 10.1128/jcm.01884-22
PMCID: PMC10281145
PMID: 37195177 [Indexed for MEDLINE]

Conflict of interest statement: The authors declare no conflict of interest.

69. **Berbamine promotes macrophage autophagy to clear Mycobacterium tuberculosis by regulating the ROS/Ca(2+) axis.**

mBio. 2023 Jun 29:e0027223. doi: 10.1128/mbio.00272-23. Online ahead of print.

Zhang S(#)(1), Zhou X(#)(2), Ou M(#)(1), Fu X(#)(1), Lin Q(3), Tao X(1), Wang Z(1), Liu A(4), Li G(1), Xu Y(3), Zhang G(1).

Drug-resistant tuberculosis (TB) poses a major threat to global TB control; consequently, there is an urgent need to develop novel anti-TB drugs or strategies. Host-directed therapy (HDT) is emerging as an effective treatment strategy, especially for drug-resistant TB. This study evaluated the effects of berbamine (BBM), a bisbenzylisoquinoline alkaloid, on mycobacterial growth in macrophages. BBM inhibited intracellular Mycobacterium tuberculosis (Mtb) growth by promoting autophagy and silencing ATG5, partially abolishing the inhibitory effect. In addition, BBM increased intracellular reactive oxygen species (ROS), while the antioxidant N-acetyl-L-cysteine (NAC) abolished BBM-induced autophagy and the ability to inhibit Mtb survival. Furthermore, the increased intracellular Ca2+ concentration induced by BBM was regulated by ROS, and BAPTA-AM, an intracellular Ca2+-chelating agent, could block ROS-mediated autophagy and Mtb clearance. Finally, BBM could inhibit the survival of drug-resistant Mtb. Collectively, these findings provide evidence that BBM, a Food and Drug Administration (FDA)-approved drug, could effectively clear drug-sensitive and -resistant Mtb through regulating ROS/Ca2+ axis-mediated

autophagy and has potential as an HDT candidate for TB therapy. IMPORTANCE It is urgent to develop novel treatment strategies against drug-resistant TB, and HDT provides a promising approach to fight drug-resistant TB by repurposing old drugs. Our studies demonstrate, for the first time, that BBM, an FDA-approved drug, not only potently inhibits intracellular drug-sensitive Mtb growth but also restricts drug-resistant Mtb by promoting macrophage autophagy. Mechanistically, BBM activates macrophage autophagy by regulating the ROS/Ca2+ axis. In conclusion, BBM could be considered as an HDT candidate and may contribute to improving the outcomes or shortening the treatment course of drug-resistant TB.

DOI: 10.1128/mbio.00272-23
PMID: 37382506

70. **Outcomes from a national screening program for Ukrainian refugees at risk of drug resistant tuberculosis in Wales.**

Thorax. 2023 Jun 21:thorax-2023-220161. doi: 10.1136/thorax-2023-220161. Online ahead of print.

Barry SM(1)(2), Davies G(3), Barry TD(4), Evans J(5), Backx M(6), Brouns M(7), Mughal A(8), Kelly S(9), Collier G(10), Ambalavanan S(11), Davies C(3), Sharp H(3), Lloyd P(9), Hester Y(12), Murray N(13), Goddard K(14), Johnstone L(8), Parry J(15), Davies O(15), Williams R(13), Ahern G(16), Smith J(16).

High rates of drug-resistant tuberculosis in Ukraine suggest screening is necessary to mitigate public health hazards for host populations. A pathway was implemented in Wales and data prospectively collected Between 8 April and 21 December 2022. Of 5425 Ukrainian arrivals, notifications were received by TB teams on 2395 (44%) of whom 1955 (82%) were screened. The refugees were young (median age 30, IQR 14-41), and predominantly female (66.1%). Interferon- gamma release assay (IGRA) tests were positive in 112 (6.5%). One Case of active tuberculosis was identified (0.05%). Our data supports European guidelines that routine screening of this population is not recommended, but we remain uncertain as to the risks of this population going forwards.

DOI: 10.1136/thorax-2023-220161
PMID: 37344177

Conflict of interest statement: Competing interests: None declared.

71. **Evaluation of Xpert MTB/RIF assay for the diagnosis of extrapulmonary**

tuberculosis in Southwest China.

PLoS Negl Trop Dis. 2023 Jun 26;17(6):e0011403. doi: 10.1371/journal.pntd.0011403. Online ahead of print.

Li TX(1), Wang J(2), Yang YS(3), Wang PS(1), Zhou G(1), Liao CY(1), Zhang HZ(1), Luo M(1), Zeng XG(4), Yang GQ(5), Yang LJ(5), Chen YK(6).

The purpose of this study was to determine the diagnostic efficacy of Xpert MTB/RIF assay for rapid diagnosis of Tuberculosis (TB) and detection of rifampicin (RIF) resistance in patients suspected of having EPTB, assessing it against traditional culture and drug susceptibility test (DST) by proportional method, and the ability to predict multidrug resistance TB by Xpert MTB/RIF assay. In this study, the Xpert MTB/RIF assay was applied to 1,614 extrapulmonary specimens. Compared with TB culture and Composite Reference Standard (CRS), the Xpert MTB/RIF assay had a high sensitivity and specificity for detection of EPTB. Depending on the culture method or CRS as the standard, sensitivity of the Xpert MTB/RIF assay for detection of MTB in pleural effusion, cerebrospinal fluid, thoracic drainage fluid and throat swabs specimens were lower than that of other specimens. According to the experimental results, we have reason to believe that Xpert MTB/RIF assay is a rapid and simple technique with high sensitivity and specificity for diagnosing EPTB and detecting drug resistance in variety of specimens. Xpert MTB/RIF assay combined with DST maybe identify more cases of multi-drug resistant tuberculosis (MDR-TB).

72. **D-Cycloserine-Induced Seizure Activity in the Emergency Department: A Case Report.**

J Pharm Pract. 2023 Jun;36(3):716-718. doi: 10.1177/08971900221074955. Epub 2022 Feb 3.

Kuhrau S(1), Boykin T(2), Rech MA(1)(2).

DOI: 10.1177/08971900221074955
PMID: 35109718 [Indexed for MEDLINE]

73. **Corrigendum to: Exposure-safety analysis of QTc interval and transaminase levels following bedaquiline administration in patients with drug-resistant tuberculosis.**

CPT Pharmacometrics Syst Pharmacol. 2023 Jun 25. doi: 10.1002/psp4.13005. Online ahead of print.

[No authors listed]

Erratum for
CPT Pharmacometrics Syst Pharmacol. 2021 Dec;10(12):1538-1549.

DOI: 10.1002/psp4.13005
PMID: 37357371

74. **Characterization of Two Novel Inhibitors of the Mycobacterium tuberculosis Cytochrome bc(1) Complex.**

Antimicrob Agents Chemother. 2023 Jun 26:e0025123. doi: 10.1128/aac.00251-23. Online ahead of print.

Gries R(#)(1)(2)(3), Dal Molin M(#)(1)(2), Chhen J(#)(1)(2), van Gumpel E(1)(2), Dreyer V(4)(5), Niemann S(4)(5), Rybniker J(1)(2)(3).

Drug-resistant tuberculosis is a global health care threat calling for novel effective treatment options. Here, we report on two novel cytochrome bc1 inhibitors (MJ-22 and B6) targeting the Mycobacterium tuberculosis respiratory chain with excellent intracellular activities in human macrophages. Both hit compounds revealed very low mutation frequencies and distinct cross-resistance patterns with other advanced cytochrome bc1 inhibitors.

DOI: 10.1128/aac.00251-23
PMID: 37358461

75. **Mechanism of mycobacterial ATP synthase inhibition by squaramides and second generation diarylquinolines.**

EMBO J. 2023 Jun 28:e113687. doi: 10.15252/embj.2023113687. Online ahead of print.

Courbon GM(1)(2), Palme PR(3), Mann L(3), Richter A(3), Imming P(3), Rubinstein JL(1)(2)(4).

Mycobacteria, such as Mycobacterium tuberculosis, depend on the activity of adenosine triphosphate (ATP) synthase for growth. The diarylquinoline bedaquiline (BDQ), a mycobacterial ATP synthase inhibitor, is an important medication for treatment of drug-resistant tuberculosis but suffers from

off-target effects and is susceptible to resistance mutations. Consequently, both new and improved mycobacterial ATP synthase inhibitors are needed. We used electron cryomicroscopy and biochemical assays to study the interaction of Mycobacterium smegmatis ATP synthase with the second generation diarylquinoline TBAJ-876 and the squaramide inhibitor SQ31f. The aryl groups of TBAJ-876 improve binding compared with BDQ, while SQ31f, which blocks ATP synthesis ~10 times more potently than ATP hydrolysis, binds a previously unknown site in the enzyme's proton-conducting channel. Remarkably, BDQ, TBAJ-876, and SQ31f all induce similar conformational changes in ATP synthase, suggesting that the resulting conformation is particularly suited for drug binding. Further, high concentrations of the diarylquinolines uncouple the transmembrane proton motive force while for SQ31f they do not, which may explain why high concentrations of diarylquinolines, but not SQ31f, have been reported to kill mycobacteria.

DOI: 10.15252/embj.2023113687
PMID: 37377118