November Literature

1. **Outcomes of Multidrug-Resistant Tuberculosis Treated With Bedaquiline or Delamanid.**

Clin Infect Dis. 2021 Oct 20;73(8):1362-1369. doi: 10.1093/cid/ciab304.

Hwang H(1), Kang H(2), Kwon YS(3), Jeon D(4), Shim TS(5), Yim JJ(1).

BACKGROUND: Since 1 September 2016, bedaquiline and delamanid have been administered for the treatment of patients with multidrug-resistant/rifampicin-resistant tuberculosis after the official approval in South Korea. This study aimed to assess and compare the final treatment outcomes of patients who received bedaquiline with those of patients who received delamanid.
METHODS: This is a nationwide cohort study of patients with multidrug-resistant/rifampicin-resistant tuberculosis in whom bedaquiline or delamanid was administered from 1 September 2016 to 28 February 2018, after receiving the official approval in South Korea. Patients were classified into the bedaquiline and delamanid group according to the first used drug. We evaluated and compared the final treatment outcomes between the groups.
RESULTS: During the study period, 284 patients with multidrug-resistant/rifampicin-resistant tuberculosis were approved to use bedaquiline or delamanid and 260 were included in the final analysis; 119 (45.8%) and 141 patients (54.2%) were classified into bedaquiline and delamanid groups, respectively. Among them, 30 patients (11.5%) exhibited additional resistance to second-line injectable drugs, 94 patients (36.2%) had additional resistance to fluoroquinolones, and 37 patients (14.2%) had resistance to both drugs. The overall treatment success rate was 79.2%. Initiation of bedaquiline rather than delamanid was not associated with treatment success (adjusted odds ratio, .671; 95% confidence interval, .350-1.285). Frequencies of adverse events were not significantly different between the 2 groups.
CONCLUSIONS: Initial choice of bedaquiline or delamanid did not make any significant difference in the final treatment outcome or the frequencies of adverse events among patients with multidrug-resistant/rifampicin-resistant tuberculosis.

DOI: 10.1093/cid/ciab304
PMID: 33837767 [Indexed for MEDLINE]

2. **The pncA gene mutations of Mycobacterium tuberculosis in multidrug-resistant tuberculosis.**

Biotechnol Appl Biochem. 2021 Nov 3. doi: 10.1002/bab.2278. Online ahead of print.

Mahmood N(1), Bhatti S(2), Abbas SN(2), Shahid S(3), Nasir SB(4).

The pncA gene encodes Pyrazinamidase enzyme which converts drug pyrazinamide to active form Pyrazinoic acid but mutations in this gene can prevent enzyme activity which leads to pyrazinamide resistance. The cross-sectional study was carried out during 2016-2017 for twelve months. The purpose of the study was to detect mutation at codon 12 and codon 85 in the pncA gene in local multidrug-resistant tuberculosis (MDR-TB) patients by developing a simple molecular test so that disease could be detected timely in the local population. DNA extracted from sputum cultured samples from multidrug-resistant tuberculosis patients and subjected to semi multiplex allele-specific PCR by using self-designed primers against the pncA gene. Among 75 samples, 53 samples were subjected to molecular analysis based on purified DNA quantity and quality. The primers produced 250 bp and 480 bp fragments indicating the mutations at codon 12 (Aspartate to Alanine) and at codon 85 (Leucine to Proline) respectively. Multidrug-resistant tuberculosis (MDR-TB) was more common in the age group 21-40 years. The 57% (n = 30) samples were found positive for pncA mutations while 43% (n = 23) showed negative results. The 13% (n = 4) samples had mutations at codon 12 in which Aspartate was converted to Alanine and they produced an amplified product of 480 bp. The 87% (n = 26) samples had mutations at codon 85 in which Leucine was converted to Proline and amplified product size was 250 bp. The mutations were simple nucleotide substitutions. The prevalence of mutations in which Leucine was substituted by Proline was higher than the mutations in which Aspartate was substituted by Alanine. A high prevalence of substitution mutation (CTG → CCG; Leucine to Proline) was detected in MDR-TB cases. Earlier detection of MDR-TB via an effective molecular diagnostic method can control the MDR tuberculosis spread in the population. This article is protected by copyright. All rights reserved.

DOI: 10.1002/bab.2278
PMID: 34731907

3. **Telomere length and mitochondrial DNA copy number in multidrug-resistant**

tuberculosis.

Tuberculosis (Edinb). 2021 Nov 10;131:102144. doi: 10.1016/j.tube.2021.102144.
Freimane L(1), Barkane L(2), Igumnova V(3), Kivrane A(3), Zole E(3), Ranka R(4).

Multidrug resistant tuberculosis (MDR-TB) is a severe disease that requires prolonged chemotherapy and is associated with an increased probability of treatment failure and death. MDR-TB is a state of heightened oxidative stress and inflammation, which could be related to the aging-related processes and immunosenescence. We, therefore, tested the hypothesis that MDR-TB is associated with alterations in aging biomarkers in peripheral blood cells. We investigated 51 MDR-TB patients and 57 healthy individuals and carried out an analysis of covariance to assess the possible impact of different variables on biomarker perturbations. The results showed that MDR-TB patients had significantly reduced telomere length (TL) and increased mitochondrial DNA copy number (mtDNA CN) ($P < 0.05$) in comparison to the controls, and MDR-TB infection was the main influencing factor. Male sex and extrapulmonary TB strongly influenced mtDNA CN increment, and MDR-TB patients with normal weight had longer telomeres than those who were underweight ($P < 0.05$). In conclusion, the evidence for shorter telomeres and higher mtDNA CN in the peripheral blood cells of MDR-TB patients was obtained indicating the connection between MDR-TB and aging biomarkers. The observed associations highlight a complicated interplay between MDR-TB and immunosenescence, thus further studies are required to achieve full understanding.

DOI: 10.1016/j.tube.2021.102144
PMID: 34781086

4. **Bedaquiline Drug Resistance Emergence Assessment in MDR-TB (DREAM): a 5-Year Prospective In-Vitro Surveillance Study of Bedaquiline and Other Second-Line Drug-Susceptibility Testing in MDR-TB Isolates.**

J Clin Microbiol. 2021 Oct 27:JCM0291920. doi: 10.1128/JCM.02919-20. Online ahead of print.

Kaniga K(1), Hasan R(2), Jou R(3), Vasiliauskienė E(4), Chuchottaworn C(5), Ismail N(6), Metchock B(7), Miliauskas S(8), Viet Nhung N(9), Rodrigues C(10), Shin S(11), Simsek H(12), Smithtikarn S(13), Ngoc ALT(9), Boonyasopun J(14), Kazi M(10), Kim S(11), Kamolwat P(13), Musteikiene G(8), Sacopon CA(15), Tahseen S(16), Vasiliauskaitė L(4), Wu MH(3), Vally Omar S(6).

Bedaquiline Drug Resistance Emergence Assessment in Multidrug-resistant-tuberculosis (MDR-TB) (DREAM) was a 5-year (2015-2019) phenotypic drug-resistance surveillance study across 11 countries. DREAM assessed the susceptibility of 5036 MDR-TB isolates of bedaquiline-treatment-naïve patients to bedaquiline and other anti-tuberculosis drugs by the 7H9 broth microdilution (BMD) and 7H10/7H11 agar dilution (AD) minimal inhibitory concentration (MIC) methods. Bedaquiline AD MIC quality control (QC) range for the H37Rv reference strain was unchanged, but the BMD MIC QC range (0.015-0.12 µg/ml) was adjusted compared with ranges from a multilaboratory, multicountry reproducibility study conforming to Clinical and Laboratory Standards Institute Tier-2 criteria. Epidemiological cut-off values of 0.12 µg/ml by BMD and 0.25 µg/ml by AD were consistent with previous bedaquiline breakpoints. An area of technical uncertainty or Intermediate category was set at 0.25 µg/ml and 0.5 µg/ml for BMD and AD, respectively. When applied to the 5036 MDR-TB isolates, bedaquiline-susceptible, intermediate and bedaquiline-resistant rates were 97.9%, 1.5% and 0.6%, respectively, for BMD, and 98.8%, 0.8% and 0.4% for AD. Resistance rates were: ofloxacin 35.1%, levofloxacin 34.2%, moxifloxacin 33.3%, 1.5% linezolid and 2% clofazimine. Phenotypic cross resistance between bedaquiline and clofazimine was 0.4% in MDR-TB and 1% in pre-extensively drug-resistant (pre-XDR-TB)/XDR-TB populations. Co-resistance to bedaquiline and linezolid, and clofazimine and linezolid, were 0.1% and 0.3%, respectively, in MDR-TB, and 0.2% and 0.4% in pre-XDR-TB/XDR-TB populations. Resistance rates to bedaquiline appear to be low in the bedaquiline-treatment-naïve population. No treatment-limiting patterns for cross-resistance and co-resistance have been identified with key TB drugs to date.

DOI: 10.1128/JCM.02919-20
PMID: 34705538

5. **Tuberculosis Pathways to Care and Transmission of Multidrug-Resistance in India.**

Am J Respir Crit Care Med. 2021 Oct 27. doi: 10.1164/rccm.202012-4333OC. Online ahead of print.

Atre SR(1), Jagtap JD(2), Faqih MI(2), Dumbare YK(2), Sawant TU(2), Ambike SL(2), Bhawalkar JS(2), Bharaswadkar SK(3), Jogewar PK(4), Adkekar RS(4), Hodgar BP(5), Jadhav V(6), Mokashi ND(7), Golub JE(8), Dixit A(9), Farhat MR(9).

RATIONALE: India is experiencing a regional increase in cases of multidrug-resistant tuberculosis (MDR-TB). Given the complexity of MDR-TB diagnosis and care, we sought to address key knowledge gaps in MDR risk factors, care delays, and drivers of delay to help guide disease control.

METHODS: From 1/2018-9/2019, we conducted interviews with adults registered with the National TB Elimination Program (NTEP) for MDR (n=128) and non-MDR-TB (n=269) treatment to quantitatively and qualitatively study care pathways. We collected treatment records and GeneXpert-TB/RIF diagnostic reports.
MEASUREMENT AND MAIN RESULTS: MDR-TB was associated with young age, and crowded residence. GeneXpert rifampicin resistance diversity was measured at 72.5% Probe E. Median time from symptom onset to diagnosis of MDR was 90 days vs. 60 days for non-MDR, Wilcoxon-P<0.01. Delay decreased by a median of 30 days among non-MDR patients with wider access to GeneXpert, Wilcoxon P=0.02. Pathways to care were complex with a median of 4 (3-5) and 3 (2-4) encounters for MDR and non-MDR respectively. Of MDR-TB patients, 68% had their first encounter in the private sector and this was associated with a larger number of subsequent healthcare encounters and catastrophic expenditure.
CONCLUSIONS: The association of MDR with young age, crowding and low genotypic diversity raise concerns of ongoing MDR transmission fueled by long delays in care. Delays are decreasing with GeneXpert use, suggesting the need for routine use in presumptive TB. Qualitatively, we identify the need to improve patient retention in the NTEP and highlight patients' trust relationship with private providers.

DOI: 10.1164/rccm.202012-4333OC
PMID: 34706203

6. **Predictors and Trends of MDR/RR-TB in Shenzhen China: A Retrospective 2012-2020 Period Analysis.**

Infect Drug Resist. 2021 Oct 27;14:4481-4491. doi: 10.2147/IDR.S335329.
eCollection 2021.

Lecai J(#)(1)(2), Mijiti P(#)(3), Chuangyue H(2), Mingzhen L(2), Qian G(3), Weiguo T(2), Jihong C(1).

PURPOSE: We analyzed the trends and predictors of multidrug-resistant (MDR) or rifampicin-resistant (RR) tuberculosis (TB) in culture-positive cases in Shenzhen during 2012-2020, after the implementation of improved strategies (scale-up molecular drug susceptibility testing [mDST], expansion of DST eligibility, and generous reimbursement of MDR-TB outpatient care costs).
MATERIALS AND METHODS: We retrospectively extracted and analyzed data from the TB Information System on drug-resistant pulmonary tuberculosis diagnosed in Shenzhen during the 2012-2020 period. We analyzed trends in RR- and MDR-TB rates in new cases during 2012-2018 and 2018-2020 periods, and among

previously-treated cases during 2012-2017 and 2017-2020 periods, using Cochran-Armitage tests. We generated multivariate logistic regression models to analyze demographic predictors of MDR/RR-TB rates.
RESULTS: We found 21,367 positive mycobacterial cultures in Shenzhen during the 2012-2020 period, and 19,951 (93.4%) were identified as Mycobacterium tuberculosis and had DST results (92.0% of those were mDST-based). Of these patients with DST results, 1630 (8.2%) were RR-TB, and 1142 (5.7%) were MDR-TB. Of the RR-TB, 70% were MDR-TB. The MDR/RR-TB rate in new TB cases increased significantly during the 2012-2018 period (P trend < 0.05), but it decreased in the 2018-2020 period (P trend > 0.05, with a significant trend for MDR-TB). Among previously treated cases, the temporal MDR/RR-TB rate trends did not differ significantly (P trend > 0.05). Our multivariate analysis showed that age younger than 30 years, housework service/unemployment, local residency, and previous TB treatment were all predictors of MDR/RR-TB. The percentage of patients with MDR-TB on treatment increased from 49.4% in 2012 to 70.5% in 2020. The treatment success rate of patients with MDR-TB during the 2012-2018 period was 71%.
CONCLUSION: During the study period in Shenzhen, the cases of MDR/RR-TB were detected, and the treatment enrollment increased and the MDR-TB rates decreased gradually after 2017. Decreasing trends may reflect the efficacy of improved strategies; however, their long-term impact on the MDR-TB burden remains to be investigated. The predictors of MDR-TB identified in our study should be considered when developing targeted MDR-TB control strategies.

DOI: 10.2147/IDR.S335329
PMCID: PMC8558316
PMID: 34737588

7. **The Treatment of Tuberculosis.**

Clin Pharmacol Ther. 2021 Dec;110(6):1455-1466. doi: 10.1002/cpt.2261. Epub 2021 Jun 5.

Peloquin CA(1), Davies GR(2)(3).

Tuberculosis (TB) remains a leading cause of infectious death worldwide, and poverty is a major driver. Clinically, TB presents as "latent" TB and active TB disease, and the treatment for each is different. TB drugs can display "early bactericidal activity (EBA)" and / or "sterilizing activity" (clearing persisters). Isoniazid is excellent at the former, and rifampin is excellent at the latter. Pyrazinamide and ethambutol complete the first-line regimen for

drug-susceptible TB, each playing a specific role. Drug-resistant TB is an increasing concern, being met, in part, with repurposed drugs (including moxifloxacin, levofloxacin, linezolid, clofazimine, and beta-lactams) and new drugs (including bedaquiline, pretomanid, and delamanid). One challenge is to select drugs without overlapping adverse drug reaction profiles. QTc interval prolongation is one such concern, but to date, it has been manageable. Drug penetration into organism sanctuaries, such as the central nervous system, bone, and pulmonary TB cavities remain important challenges. The pharmacodynamics of most TB drugs can be described by the area under the curve (AUC) divided by the minimal inhibitory concentration (MIC). The hollow fiber infection model (HFIM) and various animal models (especially mouse and macaque) allow for sophisticated pharmacokinetic/pharmacodynamic experiments. These experiments may hasten the selection of the most potent, shortest possible regimens to treat even extremely drug resistant TB. These findings can be translated to humans by optimizing drug exposure in each patient, using therapeutic drug monitoring and dose individualization.

DOI: 10.1002/cpt.2261
PMID: 33837535

8. **Risk factors associated with drug-resistant tuberculosis in Ethiopia: A systematic review and meta-analysis.**

Transbound Emerg Dis. 2021 Nov 6. doi: 10.1111/tbed.14378. Online ahead of print.

Alemu A(1)(2), Bitew ZW(3), Diriba G(1), Gumi B(2).

The emergence of drug-resistant tuberculosis (DR-TB) is becoming a challenge to the national TB control programs including Ethiopia. Different risk factors are associated with DR-TB. Identifying these risk factors in a local setting is important to strengthen the effort to prevent and control DR-TB. Thus, this study aimed to assess the risk factors associated with DR-TB in Ethiopia. The PRISMA checklist was followed to conduct this study. We systematically searched the articles from electronic databases and gray literature sources. We used the JBI tools to assess the quality of studies. Data were analyzed using STATA version 15. We estimated the pooled OR along with 95%CI. The forest plot and I2 heterogeneity test were used to assess heterogeneity among studies. We explored the presence of publication bias through visual inspection of the funnel plot and Egger's regression test. After screening 2238 articles, 27 studies were

included in the final analysis. Based on the pooled analysis of the OR, unemployment (OR; 2.71, 95%CI; 1.64, 3.78), previous TB history (OR; 4.83, 95%CI; 3.02, 6.64), contact with known TB patient (OR; 1.72, 95%CI; 1.05, 2.40), contact with the known MDR-TB patient (OR; 2.54, 95% CI; 1.46, 3.63), and having pulmonary TB (OR; 1.80, 95%CI; 1.14, 2.45) were found to be the risk factors of DR-TB. While elders (OR; 0.77, 95%CI; 0.60, 0.95) including above 45 years (OR; 0.76, 95%CI; 0.55, 0.97), and males (OR; 0.86, 95%CI; 0.76, 0.97) had lower DR-TB risk compared to their counterparts. A previous history of TB treatment is a major risk factor for acquiring DR-TB in Ethiopia that might be due to poor adherence during the first-line anti-TB treatment. Besides, having contact with a TB patient, contact with an MDR-TB patient, having pulmonary TB, and being unemployed were the risk factors of DR-TB in Ethiopia. Thus, active screening of TB contacts for DR-TB might help to detect DR-TB cases as early as possible and could help to mitigate its further transmission across the community. This article is protected by copyright. All rights reserved.

DOI: 10.1111/tbed.14378
PMID: 34741434

9. **High success and low recurrence with shorter treatment regimen for multidrug-resistant TB in Nepal.**

Public Health Action. 2021 Nov 1;11(Suppl 1):38-45. doi: 10.5588/pha.21.0041.

Koirala S(1), Shah NP(2), Pyakurel P(3), Khanal M(2), Rajbhandari SK(4), Pun T(2), Shrestha B(5), Maharjan B(2), Karki S(5), Koirala S(6), Tamang KB(7), Roggi A(8), Kumar AMV(9)(10)(11), Ortuño-Gutiérrez N(8).

SETTING: Nine drug-resistant TB centres, some of them supported by Damien Foundation in Nepal where >80% of multidrug-resistant/rifampicin-resistant TB (MDR/RR-TB) patients are treated.
OBJECTIVE: To assess the uptake, effectiveness and safety of the 9-12-month shorter treatment regimen (STR) in MDR/RR-TB patients registered from January 2018 to December 2019.
DESIGN: This was a cohort study involving secondary programme data.
RESULTS: Of 631 patients, 301 (48.0%) started and continued STR. Key reasons for ineligibility to start/continue STR were baseline resistance or exposure to second-line drugs (62.0%), contact with extensively drug-resistant TB (XDR-TB) or pre-XDR-TB (7.0%) patients and unavailability of STR drugs (6.0%). Treatment success was 79.6%; unsuccessful outcomes were death (12.0%), lost to follow-up (5.3%), failure (2.7%) and not evaluated (0.7%). Unsuccessful outcomes were

significantly associated with HIV positivity and patient age ⩾55 years, with adjusted relative risk of respectively 2.39 (95% CI 1.52-3.77) and 3.86 (95% CI 2.30-6.46). Post-treatment recurrence at 6 and 12 months was respectively 0.5% and 2.4%. Serious adverse events (SAEs) were seen in 15.3% patients - hepatotoxicity and ototoxicity were most common.
CONCLUSION: STR had a modest uptake, high treatment success and low post-treatment recurrence. For proper detection and management of SAEs, improving pharmacovigilance might be considered. Availability of rapid diagnostic test for second-line drugs is crucial for correct patient management.

DOI: 10.5588/pha.21.0041
PMCID: PMC8575385
PMID: 34778014

10. **One Step Forward: Successful End-of-Treatment Outcomes of Patients With Drug-Resistant Tuberculosis Who Received Concomitant Bedaquiline and Delamanid in Mumbai, India.**

Clin Infect Dis. 2021 Nov 2;73(9):e3496-e3504. doi: 10.1093/cid/ciaa1577.

Das M(1), Dalal A(2), Laxmeshwar C(1), Ravi S(1), Mamnoon F(1), Meneguim AC(1), Paryani R(1), Mathur T(1), Singh P(1), Mansoor H(1), Kalon S(1), Hossain FN(1), Lachenal N(3), Coutisson S(3), Ferlazzo G(4), Isaakidis P(4).

BACKGROUND: The Médecins Sans Frontières Clinic in Mumbai, India, has been providing concomitant bedaquiline (BDQ) and delamanid (DLM) in treatment regimen for patients with drug-resistant tuberculosis (DR-TB) and limited therapeutic options, referred from other healthcare institutions, since 2016. The study documents the end-of-treatment outcomes, culture-conversion rates, and serious adverse events (SAEs) during treatment.
METHODS: This was a retrospective cohort study based on routinely collected program data. In clinic, treatment regimens are designed based on culture drug sensitivity test patterns and previous drug exposures, and are provided for 20-22 months. BDQ and DLM are extended beyond 24 weeks as off-label use. Patients who initiated DR-TB treatment including BDQ and DLM (concomitantly for at least 4 weeks) during February 2016-February 2018 were included.
RESULTS: Of the 70 patients included, the median age was 25 (interquartile range [IQR], 22-32) years and 56% were females. All except 1 were fluoroquinolone resistant. The median duration of exposure to BDQ and DLM was 77 (IQR, 43-96) weeks. Thirty-nine episodes of SAEs were reported among 30 (43%) patients, including 5 instances of QTc prolongation, assessed as possibly related to BDQ

and/or DLM. The majority (69%) had culture conversion before 24 weeks of treatment. In 61 (87%), use of BDQ and DLM was extended beyond 24 weeks. Successful end-of-treatment outcomes were reported in 49 (70%) patients.
CONCLUSIONS: The successful treatment outcomes of this cohort show that regimens including concomitant BDQ and DLM for longer than 24 weeks are effective and can be safely administered on an ambulatory basis. National TB programs globally should scale up access to life-saving DR-TB regimens with new drugs.

DOI: 10.1093/cid/ciaa1577
PMID: 33079176 [Indexed for MEDLINE]

11. **Genetic Diversity and Transmission of Multidrug Resistant Mycobacterium tuberculosis strains in Lusaka, Zambia.**

Int J Infect Dis. 2021 Oct 27:S1201-9712(21)00826-2. doi: 10.1016/j.ijid.2021.10.044. Online ahead of print.

Chizimu JY(1), Solo ES(2), Bwalya P(2), Kapalamula TF(3), Akapelwa ML(3), Lungu P(4), Shrestha D(5), Fukushima Y(3), Mukonka V(6), Thapa J(3), Nakajima C(7), Suzuki Y(8).

OBJECTIVE: Zambia is among the 30 high tuberculosis burden countries in the world. Despite increasing reports of multidrug resistant tuberculosis (MDR-TB) in routine surveillance, information on the transmission of MDR Mycobacterium tuberculosis strains is largely unknown. This study elucidated genetic diversity and transmission of MDR M. tuberculosis strains in Lusaka, Zambia.
METHODS: Eighty-five MDR M. tuberculosis samples collected from the year 2013 to 2017 at the University Teaching Hospital were used. Drug-resistance associated gene sequencing, spoligotyping, 24-loci mycobacterial interspersed repetitive units-variable number of tandem repeats, and multiplex PCR for RD-Rio sub-lineage identification were applied.
RESULTS: Clades identified were LAM (48%), CAS (29%), T (14%), X (6%) and Harlem (2%). Strains belonging to SITs 21/CAS1-Kili and 20/LAM1 formed the largest clonal complexes. Combined spoligotyping and 24 loci-MIRU-VNTR revealed 47 genotypic patterns with clustering rate of 63%. Ninety five percent of LAM strains belonged to RD-Rio sub-lineage.
CONCLUSION: The high clustering rate suggested that a large proportion of MDR-TB was due to recent transmission rather than independent acquisition of MDR. This spread was attributed to clonal expansion of SIT21/CAS1-Kili and SIT20/LAM1 strains. Therefore, TB control programs recommending genotyping coupled with

conventional epidemiological methods can guide measures for stopping the spread of MDR-TB.

DOI: 10.1016/j.ijid.2021.10.044
PMID: 34718155

12. **Evaluation of the performance of the BD MAX MDR-TB test in the diagnosis of Mycobacterium tuberculosis complex in extrapulmonary and pulmonary samples.**

Expert Rev Mol Diagn. 2021 Nov 16:1-7. doi: 10.1080/14737159.2021.1997594. Online ahead of print.

Sağıroğlu P(1), Atalay MA(1).

BACKGROUND: The BD MAX MDR-TB is a recently marketed molecular test for detecting Mycobacterium tuberculosis complex (MTC), rifampin, and isoniazid drug resistance.
RESEARCH DESIGN AND METHODS: This study aimed to evaluate the BD MAX MDR-TB test performance in 933 extrapulmonary and 774 pulmonary samples.
RESULTS: Test MTC detecting sensitivity was 90.6%, 82.5%, and the specificity was 98.5%, 98.9%, in pulmonary and extrapulmonary samples, respectively. In smear-positive samples, sensitivity, and specificity were 100% for all samples. However, in smear-negative samples, the test's sensitivity and specificity were 82.3%, 98.5% in pulmonary samples, and 76.7%, 98.9% in extrapulmonary samples. Test sensitivity in detecting isoniazid resistance was 71.4%, specificity 96.8%, and in detecting rifampin resistance was 100%, specificity 93.9%, respectively.
CONCLUSIONS: BD MAX MDR-TB is a reliable, rapid, user-friendly test for detecting MTC in extrapulmonary and pulmonary samples and its resistance toward isoniazid and rifampin. It can be used as an alternative to the Xpert system assays.

DOI: 10.1080/14737159.2021.1997594
PMID: 34689662

13. **Pharmacokinetics of high-dose isoniazid in children affected by multidrug-resistant TB.**

Int J Tuberc Lung Dis. 2021 Nov 1;25(11):896-902. doi: 10.5588/ijtld.20.0870.

Winckler JL(1), Schaaf HS(1), Draper HR(1), McIlleron H(2), Norman J(2), van der

Laan LE(1), Wiesner L(2), Donald PR(1), Hesseling AC(1), Garcia-Prats AJ(1).

BACKGROUND: High-dose isoniazid (INHH) (15-20 mg/kg/day) could be administered to overcome low-level INH resistance, but pharmacokinetic data are sparse.METHODS: This observational study included South African children (<15 years) receiving INHH as preventive therapy, or treatment for multidrug-resistant TB (MDR-TB) exposure or disease. Pharmacokinetic sampling was performed after an INH dose of 20 mg/kg. Non-compartmental analysis and multivariable regression models were used to evaluate associations of key covariates with area under the curve (AUC0-24) and maximum concentration (Cmax). AUC and Cmax values were compared against proposed adult targets.RESULTS: Seventy-seven children were included, with median age of 3.7 years; 51 (66%) had MDR-TB disease and 26 (34%) had MDR-TB exposure. Five were HIV-positive, of whom four were ≥5 years old. The median AUC0-24 was 19.46 µgh/mL (IQR 10.76-50.06) and Cmax was 5.14 µg/mL (IQR 2.69-13.2). In multivariable analysis of children aged <5 years, MDR-TB disease (vs. exposure) was associated with considerably lower AUC0-24 (geometric mean ratio GMR 0.19, 95% CI 0.15-0.26; P < 0.001) and Cmax (GMR 0.20, 95% CI 0.15-0.26; P < 0.001).CONCLUSIONS: INH concentrations in children with MDR-TB disease were much lower than expected, but comparable to previous reports in children with MDR-TB exposure. Further studies should confirm these findings and explore possible causes.

DOI: 10.5588/ijtld.20.0870
PMID: 34686231 [Indexed for MEDLINE]

14. **Analysis of Factors Influencing Multidrug-Resistant Tuberculosis and Validation of Whole-Genome Sequencing in Children with Drug-Resistant Tuberculosis.**

Infect Drug Resist. 2021 Oct 24;14:4375-4393. doi: 10.2147/IDR.S331890. eCollection 2021.

Zhang Y(1), Zhao R(1), Zhang Z(1), Liu Q(1), Zhang A(1), Ren Q(1), Li S(1), Long X(1), Xu H(1).

OBJECTIVE: Pediatric tuberculosis (TB) is one of the top ten causes of death in children. Our study was to analyze influencing factors of multidrug-resistant tuberculosis (MDR-TB) and validation of whole-genome sequencing (WGS) used in children with drug-resistant TB (DR-TB).
METHODS: All Mycobacterium tuberculosis (Mtb) strains were isolated from patients aged below 18 years old of Children's Hospital of Chongqing Medical University, China. A total of 208 Mtb isolates were tested for eight anti-TB drugs with phenotypic drug susceptibility test (DST) and for genetic prediction of the susceptible profile with WGS. The patients corresponding to each strain

were grouped according to drug resistance and genotype. Influencing factors of MDR-TB and DR-TB were analyzed.
RESULTS: According to the phenotypic DST and WGS, 82.2% of Mtb strains were susceptible to all eight drugs, and 6.3% were MDR-TB. Using the phenotypic DSTs as the gold standard, the kappa value of WGS to predict isoniazid, rifampin, ethambutol, rifapentine, prothionamide, levofloxacin, moxifloxacin and amikacin was 0.84, 0.89, 0.59, 0.86, 0.89, 0.82, 0.88 and 1.00, respectively. There was significant difference in the distribution of severe TB, diagnosis, treatment and outcome between MDR and drug-susceptible group ($P<0.05$). The distribution of severe TB and treatment between DR and drug-susceptible group was statistically different ($P<0.05$). The results of binary logistic regression showed that Calmette-Guérin bacillus (BCG) vaccine is the protective factor for MDR-TB (OR=0.19), and MDR-TB is the risk factor for PTB and EPTB (OR=17.98).
CONCLUSION: The BCG vaccine is a protective factor for MDR-TB, and MDR-TB might not be confined to pulmonary infection, spreading to extrapulmonary organs in children. MDR-TB had more severe cases and a lower recovery rate than drug-susceptible TB. WGS could provide an accurate prediction of drug susceptibility test results for anti-TB drugs, which are needed for the diagnosis and precise treatment of TB in children.

DOI: 10.2147/IDR.S331890
PMCID: PMC8554314
PMID: 34729015

15. **Primary Bedaquiline Resistance Among Cases of Drug-Resistant Tuberculosis in Taiwan.**

Front Microbiol. 2021 Oct 22;12:754249. doi: 10.3389/fmicb.2021.754249. eCollection 2021.

Wu SH(1), Chan HH(1), Hsiao HC(1), Jou R(1).

Bedaquiline (BDQ), which is recommended for the treatment of drug-resistant tuberculosis (DR-TB), was introduced in Taiwan in 2014. Due to the alarming emergence of BDQ resistance, we conducted BDQ resistance analyses to strengthen our DR-TB management program. This retrospective population-based study included initial Mycobacterium tuberculosis isolates from 898 rifampicin-resistant (RR) or multidrug-resistant (MDR) TB cases never exposed to BDQ during 2008-2019. We randomly selected 65 isolates and identified 28 isolates with BDQ MIC<0.25μg/ml and MIC≥0.25μg/ml as the control and study groups, respectively. BDQ drug susceptibility testing (DST) using the MGIT960 system and Sanger sequencing of

the atpE, Rv0678, and pepQ genes was conducted. Notably, 18 isolates with BDQ MIC=0.25μg/ml, 38.9% (7/18), and 61.1% (11/18) isolates were MGIT-BDQ resistant and susceptible, respectively. Consequently, we recommended redefining MIC=0.25μg/ml as an intermediate-susceptible category to resolve discordance between different DST methods. Of the 93 isolates, 22 isolates were MGIT-BDQ-resistant and 77.3% (17/22) of MGIT-BDQ-resistant isolates harbored Rv0678 mutations. After excluding 2 MGIT-BDQ-resistant isolates with borderline resistance (GU400growth control-GU100BDQ≤1day), 100% (15/15) harbored Rv0678 gene mutations, including seven novel mutations [g-14a, Ile80Ser (N=2), Phe100Tyr, Ala102Val, Ins g 181-182 frameshift mutation (N=2), Del 11-63 frameshift mutation, and whole gene deletion (N=2)]. Since the other 22.7% (5/22) MGIT-BDQ-resistant isolates with borderline resistance (GU400growth control-GU100BDQ≤1day) had no mutation in three analyzed genes. For isolates with phenotypic MGIT-BDQ borderline resistance, checking for GU differences or conducting genotypic analyses are suggested for ruling out BDQ resistance. In addition, we observed favorable outcomes among patients with BDQ-resistant isolates who received BDQ-containing regimens regardless of Rv0678 mutations. We concluded that based on MIC≥0.25μg/ml, 3.1% (28/898) of drug-resistant TB cases without BDQ exposure showed BDQ resistance, Rv0678 was not a robust marker of BDQ resistance, and its mutations were not associated with treatment outcomes.

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PMCID: PMC8569445
PMID: 34745058

16. **Repurposing Cefazolin-Avibactam for the Treatment of Drug Resistant Mycobacterium tuberculosis.**

Front Pharmacol. 2021 Oct 22;12:776969. doi: 10.3389/fphar.2021.776969. eCollection 2021.

Srivastava S(1)(2)(3), Gumbo T(4), Thomas T(5).

Background: While tuberculosis (TB) is curable and preventable, the most effective first-line antibiotics cannot kill multi-drug resistant (MDR) Mycobacterium tuberculosis (Mtb). Therefore, effective drugs are needed to combat MDR-TB, especially in children. Our objective was to repurpose cefazolin for MDR-TB treatment in children using principles of pharmacokinetic/pharmacodynamics (PK/PD). Methods: Cefazolin minimum inhibitory concentration (MIC) was identified in 17 clinical Mtb strains, with and without combination of the β-lactamase inhibitor, avibactam. Next, dose-ranging studies

were performed using the intracellular hollow fiber model of TB (HFS-TB) to identify the optimal cefazolin exposure. Monte Carlo experiments were then performed in 10,000 children for optimal dose identification based on cumulative fraction of response (CFR) and Mtb susceptibility breakpoint in three age-groups. Results: Avibactam reduced the cefazolin MICs by five tube dilutions. Cefazolin-avibactam demonstrated maximal kill of 4.85 log10 CFU/mL in the intracellular HFS-TB over 28 days. The % time above MIC associated with maximal effect (EC80) was 46.76% (95% confidence interval: 43.04-50.49%) of dosing interval. For 100 mg/kg once or twice daily, the CFR was 8.46 and 61.39% in children <3 years with disseminated TB, 9.70 and 84.07% for 3-5 years-old children, and 17.20 and 76.13% for 12-15 years-old children. The PK/PD-derived susceptibility breakpoint was dose dependent at 1-2 mg/L. Conclusion: Cefazolin-avibactam combination demonstrates efficacy against both drug susceptible and MDR-TB clinical strains in the HFS-TB and could potentially be used to treat children with tuberculosis. Clinical studies are warranted to validate our findings.

DOI: 10.3389/fphar.2021.776969
PMCID: PMC8569112
PMID: 34744753

17. **Linezolid Population Pharmacokinetics in South African Adults with Drug-Resistant Tuberculosis.**

Antimicrob Agents Chemother. 2021 Nov 17;65(12):e0138121. doi: 10.1128/AAC.01381-21. Epub 2021 Sep 20.

Abdelwahab MT(1), Wasserman S(1)(2)(3), Brust JCM(4), Dheda K(5)(6)(7), Wiesner L(1), Gandhi NR(8)(9), Warren RM(10), Sirgel FA(10), Meintjes G(2), Maartens G(1)(2), Denti P(1).

Linezolid is widely used for drug-resistant tuberculosis (DR-TB) but has a narrow therapeutic index. To inform dose optimization, we aimed to characterize the population pharmacokinetics of linezolid in South African participants with DR-TB and explore the effect of covariates, including HIV coinfection, on drug exposure. Data were obtained from pharmacokinetic substudies in a randomized controlled trial and an observational cohort study, both of which enrolled adults with drug-resistant pulmonary tuberculosis. Participants underwent intensive and sparse plasma sampling. We analyzed linezolid concentration data using nonlinear mixed-effects modeling and performed simulations to estimate attainment of putative efficacy and toxicity targets. A total of 124

participants provided 444 plasma samples; 116 were on the standard daily dose of 600 mg, while 19 had dose reduction to 300 mg due to adverse events. Sixty-one participants were female, 71 were HIV-positive, and their median weight was 56 kg (interquartile range [IQR], 50 to 63). In the final model, typical values for clearance and central volume were 3.57 liters/h and 40.2 liters, respectively. HIV coinfection had no significant effect on linezolid exposure. Simulations showed that 600-mg dosing achieved the efficacy target (area under the concentration-time curve for the free, unbound fraction of the drug [[Formula: see text] at a MIC level of 0.5 mg/liter) with 96% probability but had 56% probability of exceeding safety target ([Formula: see text]. The 300-mg dose did not achieve adequate efficacy exposures. Our model characterized population pharmacokinetics of linezolid in South African patients with DR-TB and supports the 600-mg daily dose with safety monitoring.

DOI: 10.1128/AAC.01381-21
PMID: 34543098

18. **Determinant factors for loss to follow-up in drug-resistant tuberculosis patients: the importance of psycho-social and economic aspects.**

BMC Pulm Med. 2021 Nov 10;21(1):360. doi: 10.1186/s12890-021-01735-9.

Soedarsono S(1)(2), Mertaniasih NM(3)(4), Kusmiati T(5)(6), Permatasari A(5)(6), Juliasih NN(7)(6), Hadi C(8)(6), Alfian IN(8)(6).

BACKGROUND: Drug-resistant tuberculosis (DR-TB) is the barrier for global TB elimination efforts with a lower treatment success rate. Loss to follow-up (LTFU) in DR-TB is a serious problem, causes mortality and morbidity for patients, and leads to wide spreading of DR-TB to their family and the wider community, as well as wasting health resources. Prevention and management of LTFU is crucial to reduce mortality, prevent further spread of DR-TB, and inhibit the development and transmission of more extensively drug-resistant strains of bacteria. A study about the factors associated with loss to follow-up is needed to develop appropriate strategies to prevent DR-TB patients become loss to follow-up. This study was conducted to identify the factors correlated with loss to follow-up in DR-TB patients, using questionnaires from the point of view of patients.
METHODS: An observational study with a cross-sectional design was conducted. Study subjects were all DR-TB patients who have declared as treatment success and loss to follow-up from DR-TB treatment. A structured questionnaire was used to collect information by interviewing the subjects as respondents. Obtained data were analyzed potential factors correlated with loss to follow-up in DR-TB patients.

RESULTS: A total of 280 subjects were included in this study. Sex, working status, income, and body mass index showed a significant difference between treatment success and loss to follow-up DR-TB patients with p-value of 0.013, 0.010, 0.007, and 0.006, respectively. In regression analysis, factors correlated with increased LTFU were negative attitude towards treatment (OR = 1.2; 95% CI = 1.1-1.3), limitation of social support (OR = 1.1; 95% CI = 1.0-1.2), dissatisfaction with health service (OR = 2.1; 95% CI = 1.5-3.0)), and limitation of economic status (OR = 1.1; 95% CI = 1.0-1.2)).
CONCLUSIONS: Male patients, jobless, non-regular employee, lower income, and underweight BMI were found in higher proportion in LTFU patients. Negative attitude towards treatment, limitation of social support, dissatisfaction with health service, and limitation of economic status are factors correlated with increased LTFU in DR-TB patients. Non-compliance to treatment is complex, we suggest that the involvement and support from the combination of health ministry, labor and employment ministry, and social ministry may help to resolve the complex problems of LTFU in DR-TB patients.

DOI: 10.1186/s12890-021-01735-9
PMCID: PMC8579625
PMID: 34758794

19. **Antimycobacterial activity of acetone extract and isolated metabolites from folklore medicinal lichen Usnea laevis Nyl. against drug-sensitive and multidrug-resistant tuberculosis strains.**

J Ethnopharmacol. 2022 Jan 10;282:114641. doi: 10.1016/j.jep.2021.114641. Epub 2021 Sep 15.

Tatipamula VB(1), Annam SSP(2).

ETHNOPHARMACOLOGICAL RELEVANCE: Tuberculosis (Tb) is one of the most infectious diseases caused by Mycobacterium tuberculosis (M.t) with almost 2 million deaths yearly. Although many Tb control programs have been organised, there is an elevated number of Tb cases due to the appearance of extremely drug-resistant and multidrug-resistant (MDR) Tb strains. In the cultures of Venezuelan Andes, fruticose lichen Usnea laevis Nyl. (Usneaceae) with folklore name 'Barba de Piedra, Tusinya' is used as a natural remedy for Tb.
AIM OF THE STUDY: This study was performed to provide a scientific rationale for the folklore usage of U. laevis in treating Tb by validating its antimycobacterial activity against two drug-sensitive and four MDR-Tb strains.
MATERIALS AND METHODS: The mycobacterial inhibitory activities of acetone

extract (Ul), fractions (F1-10), and isolated metabolites (1-4) of U. laevis were evaluated against M.t H37Ra using 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide reduction menadione assay (XRMA). Furthermore, Ul and 1-4 were subjected to antimycobacterial activity against M.t H37Ra, Mycobacterium smegmatis, and four MDR-Tb (MDR-A8, MDR-V791, MDR-R and MDR-40) strains using resazurin microtitre plate assay (REMA) and cytotoxicity against THP-1 macrophages using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and their selectivity index values were also calculated.
RESULTS: Initially, Ul has shown prominent inhibitory activity (IC50 value: 5.44 ± 0.36 µg/ml) and four of its fractions (F1, F2, F5 and F7) also exhibited the best inhibitory activity (IC50 values ranged from 7.46 ± 0.19 to 71.38 ± 2.57 µg/ml) against M.t H37Ra using XRMA. Purification of these bioactive fractions identified four metabolites, namely usnic acid (1), atranorin (2), salazinic acid (3), and lobaric acid (4). From the MIC values of REMA, it was identified that Ul, 1 and 4 were more effective in inhibiting the growth of all four MDR-Tb strains, compared to first-line drug rifampicin. Interestingly, Ul has shown better antimycobacterial activity than 1-4 and rifampicin against MDR-Tb strains may be due to the synergistic effect of its metabolites. Also, the IC50 values of Ul and 1-4 on THP-1 macrophages were found to be far higher than MIC values against tested Tb strains, indicating that THP-1 macrophages were not harmfully affected at concentrations that were effective against Tb strains. Further, the calculated selectivity index values revealed the more active and non-toxicity of Ul, 1 and 4 against MDR-Tb strains than rifampicin.
CONCLUSIONS: The current study lends the first evidence for the presence of antimycobacterial metabolites in U. laevis. The results exposed the Andean folklore use of U. laevis for treating Tb, and the key biomarker metabolites were found to be 1 and 4. Hence, it can be concluded that U. laevis can be used as a potential source for the novel drug development for MDR-Tb.

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PMID: 34536516

20. **Genotypic Resistance of Pyrazinamide but Not Minimum Inhibitory Concentration Is Associated With Longer Time to Sputum Culture Conversion in Patients With Multidrug-resistant Tuberculosis.**

Clin Infect Dis. 2021 Nov 2;73(9):e3511-e3517. doi: 10.1093/cid/ciaa1509.

Kuhlin J(1)(2), Davies Forsman L(1)(2), Mansjö M(3), Jonsson Nordvall M(4),

Wijkander M(3), Wagrell C(2), Jonsson J(5), Groenheit R(3), Werngren J(3), Schön T(6)(7), Bruchfeld J(1)(2).

BACKGROUND: Pyrazinamide (PZA) resistance in multidrug-resistant tuberculosis (MDR-TB) is common; yet, it is not clear how it affects interim and treatment outcomes. Although rarely performed, phenotypic drug susceptibility testing (pDST) is used to define PZA resistance, but genotypic DST (gDST) and minimum inhibitory concentration (MIC) could be beneficial. We aimed to assess the impact of PZA gDST and MIC on time to sputum culture conversion (SCC) and treatment outcome in patients with MDR-TB.
METHODS: Clinical, microbiological, and treatment data were collected in this cohort study for all patients diagnosed with MDR-TB in Sweden from 1992-2014. MIC, pDST, and whole-genome sequencing of the pncA, rpsA, and panD genes were used to define PZA resistance. A Cox regression model was used for statistical analyses.
RESULTS: Of 157 patients with MDR-TB, 56.1% (n = 88) had PZA-resistant strains and 49.7% (n = 78) were treated with PZA. In crude and adjusted analysis (hazard ratio [HR], 0.49; 95% conficence interval [CI], .29-.82; P = .007), PZA gDST resistance was associated with a 29-day longer time to SCC. A 2-fold decrease in dilutions of PZA MIC for PZA-susceptible strains showed no association with SCC in crude or adjusted analyses (HR, 0.98; 95% CI, .73-1.31; P = .89). MIC and gDST for PZA were not associated with treatment outcome.
CONCLUSIONS: In patients with MDR-TB, gDST PZA resistance was associated with a longer time to SCC. Rapid PZA gDST is important to identify patients who may benefit from PZA treatment.

DOI: 10.1093/cid/ciaa1509
PMID: 33011791 [Indexed for MEDLINE]

21. **Drug exposure and susceptibility of second-line drugs correlate with treatment response in patients with multidrug-resistant tuberculosis: a multi-centre prospective cohort study in China.**

Eur Respir J. 2021 Nov 11:2101925. doi: 10.1183/13993003.01925-2021. Online ahead of print.

Zheng X(1), Davies Forsman L(2)(3), Bao Z(4), Xie Y(5), Ning Z(5), Schön T(6)(7), Bruchfeld J(2)(3), Xu B(1), Alffenaar JW(8)(9)(10), Hu Y(11).

BACKGROUND: Understanding the impact of drug exposure and susceptibility on treatment response of multidrug-resistant tuberculosis (MDR-TB) will help to optimize treatment. This study aimed to investigate the association between drug exposure, susceptibility and response to MDR-TB treatment.
METHODS: Drug exposure and susceptibility for second-line drugs were measured for patients with MDR-TB. Multivariate analysis was applied to investigate the impact of drug exposure and susceptibility on sputum culture conversion and treatment outcome. Probability of target attainment was evaluated. Random Forest and classification and regression tree (CART) analysis was used to identify key predictors and their clinical targets among patients on WHO-recommended regimens.
RESULTS: Drug exposure and corresponding susceptibility were available for 197 patients with MDR-TB. Target attainment was highly variable ranging from 0% for ethambutol to 97% for linezolid, while patients with fluoroquinolones above targets had higher probability of two-month culture conversion (56.3% versus 28.6%, OR 2.91, 95% CI 1.42-5.94) and favourable outcome (88.8% versus 68.8%, OR 2.89, 95% CI 1.16-7.17). Higher exposure values of fluoroquinolones, linezolid and pyrazinamide were associated with earlier sputum culture conversion. CART analysis selected moxifloxacin AUC/MIC of 231 and linezolid AUC/MIC of 287 as best predictors for six-month culture conversion in patients receiving identical Group A-based regimen. These association were confirmed in multivariate analysis.
CONCLUSIONS: Our findings indicated that target attainment of TB drugs is associated with response to treatment. The CART-derived thresholds may serve as targets for early dose adjustment in a future randomized controlled study to improve the MDR-TB treatment outcome.

DOI: 10.1183/13993003.01925-2021
PMID: 34737224

22. **A Comprehensive Evaluation of GeneLEAD VIII DNA Platform Combined to Deeplex Myc-TB(®) Assay to Detect in 8 Days Drug Resistance to 13 Antituberculous Drugs and Transmission of Mycobacterium tuberculosis Complex Directly From Clinical Samples.**

Front Cell Infect Microbiol. 2021 Oct 29;11:707244. doi: 10.3389/fcimb.2021.707244. eCollection 2021.

Bonnet I(1)(2)(3), Enouf V(4), Morel F(1)(2)(3), Ok V(1)(2)(3), Jaffré J(1)(2)(3), Jarlier V(1)(2), Aubry A(1)(2)(3), Robert J(1)(2)(3), Sougakoff

W(1)(2)(3).

The GeneLEAD VIII (Diagenode, Belgium) is a new, fully automated, sample-to-result precision instrument for the extraction of DNA and PCR detection of Mycobacterium tuberculosis complex (MTBC) directly from clinical samples. The Deeplex Myc-TB® assay (Genoscreen, France) is a diagnostic kit based on the deep sequencing of a 24-plexed amplicon mix allowing simultaneously the detection of resistance to 13 antituberculous (antiTB) drugs and the determination of spoligotype. We evaluated the performance of a strategy combining the both mentioned tools to detect directly from clinical samples, in 8 days, MTBC and its resistance to 13 antiTB drugs, and identify potential transmission of strains from patient-to-patient. Using this approach, we screened 112 clinical samples (65 smear-negative) and 94 MTBC cultured strains. The sensitivity and the specificity of the GeneLEAD/Deeplex Myc-TB approach for MTBC detection were 79.3% and 100%, respectively. One hundred forty successful Deeplex Myc-TB results were obtained for 46 clinical samples and 94 strains, a total of 85.4% of which had a Deeplex Myc-TB susceptibility and resistance prediction consistent with phenotypic drug susceptibility testing (DST). Importantly, the Deeplex Myc-TB assay was able to detect 100% of the multidrug-resistant (MDR) MTBC tested. The lowest concordance rates were for pyrazinamide, ethambutol, streptomycin, and ethionamide (84.5%, 81.5%, 73%, and 55%, respectively) for which the determination of susceptibility or resistance is generally difficult with current tools. One of the main difficulties of Deeplex Myc-TB is to interpret the non-synonymous uncharacterized variants that can represent up to 30% of the detected single nucleotide variants. We observed a good level of concordance between Deeplex Myc-TB-spoligotyping and MIRU-VNTR despite a lower discriminatory power for spoligotyping. The median time to obtain complete results from clinical samples was 8 days (IQR 7-13) provided a high-throughput NGS sequencing platform was available. Our results highlight that the GeneLEAD/Deeplex Myc-TB approach could be a breakthrough in rapid diagnosis of MDR TB in routine practice.

DOI: 10.3389/fcimb.2021.707244
PMCID: PMC8586210
PMID: 34778100

23. **Gender differences among patients with drug resistant tuberculosis and HIV co-infection in Uganda: a countrywide retrospective cohort study.**

BMC Infect Dis. 2021 Oct 24;21(1):1093. doi: 10.1186/s12879-021-06801-5.

Baluku JB(1)(2), Mukasa D(3), Bongomin F(4), Stadelmann A(5), Nuwagira E(6), Haller S(7), Ntabadde K(8), Turyahabwe S(9).

BACKGROUND: Gender differences among patients with drug resistant tuberculosis (DRTB) and HIV co-infection could affect treatment outcomes. We compared characteristics and treatment outcomes of DRTB/HIV co-infected men and women in Uganda.
METHODS: We conducted a retrospective chart review of patients with DRTB from 16 treatment sites in Uganda. Eligible patients were aged ≥ 18 years, had confirmed DRTB, HIV co-infection and a treatment outcome registered between 2013 and 2019. We compared socio-demographic and clinical characteristics and tuberculosis treatment outcomes between men and women. Potential predictors of mortality were determined by cox proportional hazard regression analysis that controlled for gender. Statistical significance was set at $p < 0.05$.
RESULTS: Of 666 DRTB/HIV co-infected patients, 401 (60.2%) were men. The median (IQR) age of men and women was 37.0 (13.0) and 34.0 (13.0) years respectively ($p < 0.001$). Men were significantly more likely to be on tenofovir-based antiretroviral therapy (ART), high-dose isoniazid-containing DRTB regimen and to have history of cigarette or alcohol use. They were also more likely to have multi-drug resistant TB, isoniazid and streptomycin resistance and had higher creatinine, aspartate and gamma-glutamyl aminotransferase and total bilirubin levels. Conversely, women were more likely to be unemployed, unmarried, receive treatment from the national referral hospital and to have anemia, a capreomycin-containing DRTB regimen and zidovudine-based ART. Treatment success was observed among 437 (65.6%) and did not differ between the genders. However, mortality was higher among men than women (25.7% vs. 18.5%, $p = 0.030$) and men had a shorter mean (standard error) survival time (16.8 (0.42) vs. 19.0 (0.46) months), Log Rank test ($p = 0.046$). Predictors of mortality, after adjusting for gender, were cigarette smoking (aHR = 4.87, 95% CI 1.28-18.58, $p = 0.020$), an increase in alanine aminotransferase levels (aHR = 1.05, 95% CI 1.02-1.07, $p < 0.001$), and history of ART default (aHR = 3.86, 95% CI 1.31-11.37, $p = 0.014$) while a higher baseline CD4 count was associated with lower mortality (aHR = 0.94, 95% CI 0.89-0.99, $p = 0.013$ for every 10 cells/mm3 increment).
CONCLUSION: Mortality was higher among men than women with DRTB/HIV co-infection which could be explained by several sociodemographic and clinical differences.

DOI: 10.1186/s12879-021-06801-5
PMCID: PMC8542192
PMID: 34689736 [Indexed for MEDLINE]

24. **Novel 2-Nitroimidazole and imidazooxazole Derivatives and Their Activity Against Trypanosoma cruzi and Mycobacterium tuberculosis.**

Med Chem. 2021 Nov 16. doi: 10.2174/1573406418666211116144952. Online ahead of print.

Faria JV(1), Passos FPZ(1), da Costa PHA(1), de Oliveira AP(1), da Cruz YOD(1), Castelo-Branco FS(1), Lourenço MCS(2), Murta SMF(3), Junior PAS(3), Bernardino AMR(4), Bastos MM(1), Boechat N(1).

BACKGROUND: Tuberculosis (TB) is one of the top ten causes of death worldwide, while Chagas disease (CD) is the parasitic disease that kills the largest number of people in the Americas. TB is the leading cause of death for patients with AIDS; it kills 1.5 million people and causes 10 million new cases every year. The lack of newly developed chemotherapeutic agents and insufficient access to health care services for a diagnosis increase the incidence of multidrug-resistant TB (MDRTB) cases. Although CD was identified in 1909, the chronic stages of the disease still lack adequate treatment.
OBJECTIVE: The purpose of this work was to design and synthesize two new series of 2-nitroimidazole 5a-e and imidazooxazoles 6a-e with 1H-1,2,3-triazolil nucleus and evaluate their activities against Tc and Mycobacterium tuberculosis (Mtb).
METHODS: Two series of five compounds were synthesized in a 3 or 4-step route in moderated yields, and their structures were confirmed by NMR spectral data analyses. The in vitro antitrypanosomal evaluation of products was carried out in an intracellular model using L929 cell line infected with trypomastigotes and amastigote forms of Tc of β-galactosidase-transfected Tulahuen strain. Their antimycobacterial activity was evaluated against Mtb strain H37Rv.
RESULTS: In general, 2-nitroimidazolic derivatives proved to be more potent in regard to antitrypanocidal and antimycobacterial activity. The non-cytotoxic 2-nitroimidazole derivative 5b was the most promising with a half maximum inhibitory concentration of 3.2 μM against Tc and a minimum inhibitory concentration of 65.3 μM against Mtb.
CONCLUSION: Our study reinforced the importance of 2-nitroimidazole and 1H-1,2,3-triazole nuclei in antimicrobial activity. In addition, derivative 5b proved to be the most promising, presenting important activity against Tc and Mtb and could be used as a starting point for the development of new agents against these diseases.

DOI: 10.2174/1573406418666211116144952
PMID: 34784878

25. **Transmission of multidrug-resistant tuberculosis in Shimen community in Shanghai, China: a molecular epidemiology study.**

BMC Infect Dis. 2021 Oct 29;21(1):1118. doi: 10.1186/s12879-021-06725-0.

Han Z(#)(1), Li J(#)(2), Sun G(3), Gu K(3), Zhang Y(2), Yao H(4), Jiang Y(5).

BACKGROUND: Multidrug-resistant tuberculosis (MDR-TB) has become a major public health problem in China, with mounting evidence suggesting that recent transmission accounts for the majority of MDR-TB. Here we aimed to reveal the transmission pattern of an MDR-TB outbreak in the Jing'an District of Shanghai between 2010 and 2015.
METHODS: We used whole-genome sequencing (WGS) to conduct genomic clustering analysis along with field epidemiological investigation to determine the transmission pattern and drug resistance profile of a cluster with ten MDR-TB patients in combining field epidemiological investigation.
RESULTS: The ten MDR-TB patients with genotypically clustered Beijing lineage strains lived in a densely populated, old alley with direct or indirect contact history. The analysis of genomic data showed that the genetic distances of the ten strains (excluding drug-resistant mutations) were 0-20 single nucleotide polymorphisms (SNPs), with an average distance of 9 SNPs, suggesting that the ten MDR-TB patients were infected and developed the onset of illness by the recent transmission of M. tuberculosis. The genetic analysis confirmed definite epidemiological links between the clustered cases.
CONCLUSIONS: The integration of the genotyping tool in routine tuberculosis surveillance can play a substantial role in the detection of MDR-TB transmission events. The leverage of genomic analysis in combination with the epidemiological investigation could further elucidate transmission patterns. Whole-genome sequencing could be integrated into intensive case-finding strategies to identify missed cases of MDR-TB and strengthen efforts to interrupt transmission.

DOI: 10.1186/s12879-021-06725-0
PMCID: PMC8557015
PMID: 34715793 [Indexed for MEDLINE]

26. **Novel candidates in the clinical development pipeline for TB drug development and their synthetic approaches.**

Chem Biol Drug Des. 2021 Nov;98(5):787-827. doi: 10.1111/cbdd.13934. Epub 2021 Sep 16.

Kumar A(1), Karkara BB(1), Panda G(1).

Tuberculosis (TB) is an infection caused by Mycobacterium tuberculosis (Mtb) and one of the deadliest infectious diseases in the world. Mtb has the ability to become dormant within the host and to develop resistance. Hence, new antitubercular agents are required to overcome problems in the treatment of multi-drug-resistant Tb (MDR-Tb) and extensively drug-resistant Tb (XDR-Tb) along with shortening the treatment time. Several efforts are being made to develop very effective new drugs for Tb, within the pharmaceutical industry, the academia and through public-private partnerships. This review will address the antitubercular activities, biological target, mode of action, synthetic approaches and thoughtful concept for the development of several new drugs currently in the clinical trial pipeline (up to October 2019) for tuberculosis. The aim of this review may be very useful in scheming new chemical entities (NCEs) for Mtb.

DOI: 10.1111/cbdd.13934
PMID: 34397161

27. **Assessment of epidemiological and genetic characteristics and clinical outcomes of resistance to bedaquiline in patients treated for rifampicin-resistant tuberculosis: a cross-sectional and longitudinal study.**

Lancet Infect Dis. 2021 Nov 12:S1473-3099(21)00470-9. doi: 10.1016/S1473-3099(21)00470-9. Online ahead of print.

Ismail NA(1), Omar SV(2), Moultrie H(3), Bhyat Z(3), Conradie F(4), Enwerem M(5), Ferreira H(6), Hughes J(7), Joseph L(3), Kock Y(8), Letsaolo V(3), Maartens G(9), Meintjes G(9), Ngcamu D(3), Okozi N(3), Padanilam X(10), Reuter A(11), Romero R(12), Schaaf S(7), Te Riele J(13), Variava E(14), van der Meulen M(3), Ismail F(15), Ndjeka N(8).

BACKGROUND: Bedaquiline improves outcomes of patients with rifampicin-resistant and multidrug-resistant (MDR) tuberculosis; however, emerging resistance threatens this success. We did a cross-sectional and longitudinal analysis evaluating the epidemiology, genetic basis, and treatment outcomes associated with bedaquiline resistance, using data from South Africa (2015-19).
METHODS: Patients with drug-resistant tuberculosis starting bedaquiline-based

treatment had surveillance samples submitted at baseline, month 2, and month 6, along with demographic information. Culture-positive baseline and post-baseline isolates had phenotypic resistance determined. Eligible patients were aged 12 years or older with a positive culture sample at baseline or, if the sample was invalid or negative, a sample within 30 days of the baseline sample submitted for bedaquiline drug susceptibility testing. For the longitudinal study, the first surveillance sample had to be phenotypically susceptible to bedaquiline for inclusion. Whole-genome sequencing was done on bedaquiline-resistant isolates and a subset of bedaquiline-susceptible isolates. The National Institute for Communicable Diseases tuberculosis reference laboratory, and national tuberculosis surveillance databases were matched to the Electronic Drug-Resistant Tuberculosis Register. We assessed baseline resistance prevalence, mutations, transmission, cumulative resistance incidence, and odds ratios (ORs) associating risk factors for resistance with patient outcomes.
FINDINGS: Between Jan 1, 2015, and July 31, 2019, 8041 patients had surveillance samples submitted, of whom 2023 were included in the cross-sectional analysis and 695 in the longitudinal analysis. Baseline bedaquiline resistance prevalence was 3·8% (76 of 2023 patients; 95% CI 2·9-4·6), and it was associated with previous exposure to bedaquiline or clofazimine (OR 7·1, 95% CI 2·3-21·9) and with rifampicin-resistant or MDR tuberculosis with additional resistance to either fluoroquinolones or injectable drugs (pre-extensively-drug resistant [XDR] tuberculosis: 4·2, 1·7-10·5) or to both (XDR tuberculosis: 4·8, 2·0-11·7). Rv0678 mutations were the sole genetic basis of phenotypic resistance. Baseline resistance could be attributed to previous bedaquiline or clofazimine exposure in four (5·3%) of 76 patients and to primary transmission in six (7·9%). Odds of successful treatment outcomes were lower in patients with baseline bedaquiline resistance (0·5, 0·3-1). Resistance during treatment developed in 16 (2·3%) of 695 patients, at a median of 90 days (IQR 62-195), with 12 of these 16 having pre-XDR or XDR.
INTERPRETATION: Bedaquiline resistance was associated with poorer treatment outcomes. Rapid assessment of bedaquiline resistance, especially when patients were previously exposed to bedaquiline or clofazimine, should be prioritised at baseline or if patients remain culture-positive after 2 months of treatment. Preventing resistance by use of novel combination therapies, current treatment optimisation, and patient support is essential.
FUNDING: National Institute for Communicable Diseases of South Africa.

DOI: 10.1016/S1473-3099(21)00470-9
PMID: 34780706

28. **Rifampicin and isoniazid drug resistance among patients diagnosed with pulmonary**

tuberculosis in southwestern Uganda.

PLoS One. 2021 Oct 29;16(10):e0259221. doi: 10.1371/journal.pone.0259221. eCollection 2021.

Micheni LN(1)(2), Kassaza K(1), Kinyi H(3), Ntulume I(2), Bazira J(1).

Multidrug-resistant tuberculosis (MDR-TB) has become a major threat to the control of tuberculosis globally. Uganda is among the countries with a relatively high prevalence of tuberculosis despite significant control efforts. In this study, the drug resistance of Mycobacterium tuberculosis to rifampicin (RIF) and isoniazid (INH) was investigated among patients diagnosed with pulmonary tuberculosis in Southwestern Uganda. A total of 283 sputum samples (266 from newly diagnosed and 17 from previously treated patients), collected between May 2018 and April 2019 at four different TB diagnostic centres, were assessed for RIF and INH resistance using high-resolution melt curve analysis. The overall prevalence of monoresistance to INH and RIF was 8.5% and 11% respectively, while the prevalence of MDR-TB was 6.7%. Bivariate analysis showed that patients aged 25 to 44 years were at a higher risk of developing MDR-TB (cOR 0.253). Furthermore, among the newly diagnosed patients, the prevalence of monoresistance to INH, RIF and MDR-TB was 8.6%, 10.2% and 6.4% respectively; while among the previously treated cases, these prevalence rates were 5.9%, 23.5% and 11.8%. These rates are higher than those reported previously indicating a rise in MTB drug resistance and may call for measures used to prevent a further rise in drug resistance. There is also a need to conduct frequent drug resistance surveys, to monitor and curtail the development and spread of drug-resistant TB.

DOI: 10.1371/journal.pone.0259221
PMCID: PMC8555815
PMID: 34714879

29. **Magnitude of Multidrug Resistance and Associated Factors of Pulmonary Tuberculosis Among Adult Smear Positive Patients in Eastern Ethiopia.**

Infect Drug Resist. 2021 Oct 28;14:4493-4500. doi: 10.2147/IDR.S326798. eCollection 2021.

Amin Z(1), Mitiku H(2), Marami D(2), Shume T(2), Weldegebreal F(2).

BACKGROUND: In Ethiopia, multidrug resistant tuberculosis is a major public health problem. However, information is scarce regarding MDR-TB and associated factors.

OBJECTIVE: The study was aimed to assess the magnitude of multidrug resistance and associated factors of pulmonary tuberculosis among adult smear-positive patients in Harari regional state health facilities, eastern Ethiopia.
METHODS: A cross-sectional study was conducted among 395 adult smear-positive pulmonary tuberculosis patients attending health facilities from March to October 2019. Smear-positive sputum samples were collected from health facilities, and transported to Harari Health Research and Regional Laboratory, and tested for drug susceptibility using a line probe assay. Data were analyzed using Statistical Package for Social Sciences version 20. Bivariate and multivariable logistic regression analyses with 95% confidence intervals were carried out to identify factors associated with multidrug-resistant tuberculosis.
RESULTS: The overall magnitude of multidrug-resistant tuberculosis was 3.8% (15/395) (95% CI: 2.0-5.8%). Being male (AOR = 4.9; 95% CI: 1.16, 20.5), patients with a previous history of tuberculosis (AOR = 4.9; 95% CI: 1.5, 29.6), treatment failure (AOR = 8.5; 95% CI: 1.61, 45.3), treatment default (AOR = 10.38; 95% CI: 1.86, 58.0), human immunodeficiency virus co-infection (AOR = 9.83.95% CI: 3, 21, 30.1) and a previous history of contact with multidrug-resistant tuberculosis patients (AOR = 14.4; 95% CI: 3.1, 67.6) had higher odds of multidrug-resistant tuberculosis.
CONCLUSION: The overall magnitude of multidrug-resistant tuberculosis was high. Strengthening the tuberculosis control program by giving special attention to HIV co-infected patients, treatment failure and default, previously infected patients as well as to those individuals who have a history of contact with multidrug-resistant tuberculosis infected patients .

DOI: 10.2147/IDR.S326798
PMCID: PMC8560056
PMID: 34737589

30. **Outcome of drug resistant tuberculosis in Indian children.**

Trop Doct. 2021 Nov 18:494755211043852. doi: 10.1177/00494755211043852. Online ahead of print.

Shetty NS(1), Bodhanwala M(2), Shah I(1).

We aimed to determine the outcome of bacteriologically confirmed drug-resistant (DR) tuberculosis (TB) in 174 children. We found that DR-TB infected children have nonetheless a high treatment completion rate with a low incidence of fatality and treatment failure. Reversible adverse drug reactions are common

during therapy.

DOI: 10.1177/00494755211043852
PMID: 34791934

31. **Reduced Susceptibility of Mycobacterium tuberculosis to Bedaquiline During Antituberculosis Treatment and Its Correlation With Clinical Outcomes in China.**

Clin Infect Dis. 2021 Nov 2;73(9):e3391-e3397. doi: 10.1093/cid/ciaa1002.

Liu Y(1), Gao M(2), Du J(1), Wang L(3), Gao J(1), Shu W(1), Wang Y(4), Xue Z(4), Li L(1), Pang Y(3)(4).

BACKGROUND: We aimed to assess the proportion of multidrug-resistant tuberculosis (MDR-TB) cases with initial bedaquiline (BDQ) resistance, monitor the dynamics of BDQ susceptibility of Mycobacterium tuberculosis isolates during therapy, and correlate susceptibility with MDR-TB patient clinical outcomes in China.
METHODS: A retrospective, cohort study of MDR-TB patients was conducted, with positive cultures collected from cases at 13 sites. Patients with nontuberculous mycobacterial infection during anti-TB therapy were excluded. BDQ minimal inhibitory concentrations (MICs) were determined using a 7H9 Middlebrook broth-based microdilution method. Mutations that conferred BDQ resistance were detected via Sanger sequencing.
RESULTS: A total of 277 patients receiving BDQ treatment were studied, with BDQ resistance noted in isolates from 2.2% (6/277) of MDR-TB cases, sputum conversion observed in 5 cases, and culture conversion observed in 138 cases within 2 weeks. Another 15 and 30 isolates were excluded from final analysis due to failures in obtaining subcultures and serial isolates, respectively. Of 94 cases that yielded serial isolates, 11 exhibited reduced BDQ susceptibility, while 3 of 5 cases with acquired resistance failed to culture-convert. Sequence analysis revealed that 6 of 11 BDQ-resistant isolates harbored Rv0678 mutations; no mutations were detected in 3 other BDQ resistance-associated genes. No significant intergroup difference in culture conversion time was observed.
CONCLUSIONS: MDR-TB patients in China exhibited a low initial BDQ resistance rate. MDR-TB cases with acquired BDQ resistance were at greater risk of treatment failure relative to initially BDQ-resistant cases. Rv0678 mutations accounted for BDQ resistance in this cohort.

DOI: 10.1093/cid/ciaa1002
PMID: 32667984 [Indexed for MEDLINE]

32. **Mycobacterium Tuberculosis Peritonitis in Peritoneal Dialysis Patients: A Scoping Review.**

Nephrology (Carlton). 2021 Nov 7. doi: 10.1111/nep.13997. Online ahead of print.

Thomson Benjamin KA(1)(2), Stephen V(3), Bogdan M(1).

BACKGROUND: The clinical syndrome of Mycobacterium tuberculosis (TB) peritoneal dialysis (PD) peritonitis is poorly understood. Whether local TB patterns modify the clinical syndrome, and what factors associate with poor outcomes is also unknown.
METHODS: A scoping review identified published cases of TB PD peritonitis. Cases from low and high TB burden areas were compared, and cases that did or did not suffer a poor clinical outcome were compared.
RESULTS: There were 216 cases identified. Demographics, presentation, diagnosis, treatment and outcomes were described. Significant delays in diagnosis were common (6.1 weeks) and were longer in patients from low TB burden regions (7.3 vs 3.7 weeks). In low TB burden areas, slower diagnostic methods were more commonly used like PD fluid culture (64.3 vs. 32.7%), and treatment was less likely with quinolone antibiotics (6.9 vs 34.1%). Higher national TB incidence and lower GDP per capita were found in cases that suffered PD catheter removal or death. Diagnostic delays were not longer in cases in which a patient suffered PD catheter removal or death. Cases that suffered death were older (51.9 vs 45.1 years) and less likely female (37.8 vs. 55.7%). Removal of PD catheter was more common in cases in which a patient died (62.0 vs 49.1%).
CONCLUSIONS: Outcomes in TB PD peritonitis are best predicted by national TB incidence, patient age and sex. Several unique features are identified to alert clinicians to use more rapid diagnostic methods that might enhance outcomes in TB PD peritonitis. This article is protected by copyright. All rights reserved.

DOI: 10.1111/nep.13997
PMID: 34743395

33. **Comparative Efficacy of the Novel Diarylquinoline TBAJ-876 and Bedaquiline against a Resistant Rv0678 Mutant in a Mouse Model of Tuberculosis.**

Antimicrob Agents Chemother. 2021 Nov 17;65(12):e0141221. doi:

10.1128/AAC.01412-21. Epub 2021 Sep 27.

Almeida D(1), Converse PJ(1), Li SY(1), Upton AM(2), Fotouhi N(2), Nuermberger EL(1)(3).

Bedaquiline (BDQ, B) is the first-in-class diarylquinoline to be approved for treatment of tuberculosis (TB). Recent guidelines recommend its use in treatment of multidrug- and extensively drug-resistant tuberculosis (MDR/XDR-TB). The newly approved regimen combining BDQ with pretomanid and linezolid is the first 6-month oral regimen proven to be effective against MDR/XDR-TB. However, the emergence of BDQ resistance, primarily due to inactivating mutations in the Rv0678 gene encoding a repressor of the MmpS5-MmpL5 transporter, threatens to undermine the efficacy of new BDQ-containing regimens. Since the shift in MIC due to these mutations is relatively small (2-8×), safer, and more potent, diarylquinoline analogues may be more effective than BDQ. TBAJ-876, which is in phase 1 trials, has more potent in vitro activity and a superior pre-clinical safety profile than BDQ. Using a murine model of TB, we evaluated the dose-dependent activity of TBAJ-876 compared to BDQ against the wild-type H37Rv strain and an isogenic Rv0678 loss-of-function mutant. Although the mutation affected the MIC of both drugs, the MIC of TBAJ-876 against the mutant was 10-fold lower than that of BDQ. TBAJ-876 at doses ≥6.25 mg/kg had greater efficacy against both strains compared to BDQ at 25 mg/kg, when administered alone or in combination with pretomanid and linezolid. Likewise, no selective amplification of BDQ-resistant bacteria was observed at TBAJ-876 doses ≥6.25 mg/kg. These results indicate that replacing BDQ with TBAJ-876 may shorten the duration of TB treatment and be more effective in treating and preventing infections caused by Rv0678 mutants.

DOI: 10.1128/AAC.01412-21
PMID: 34570644

34. **Correlating genetic mutations with isoniazid phenotypic levels of resistance in Mycobacterium tuberculosis isolates from patients with drug-resistant tuberculosis in a high burden setting.**

Eur J Clin Microbiol Infect Dis. 2021 Dec;40(12):2551-2561. doi: 10.1007/s10096-021-04316-0. Epub 2021 Jul 23.

Pinhata JMW(1), Brandao AP(2)(3), Mendes FF(2), Rabello MCDS(4), Ferrazoli L(2), de Oliveira RS(2).

We analysed mutations in katG, inhA and rpoB genes, and isoniazid phenotypic resistance levels in Mycobacterium tuberculosis isolates from drug-resistant TB

patients from São Paulo state, Brazil. Isolates resistant to the critical concentration of isoniazid in MGIT (0.1 µg/mL) were screened for mutations in katG 315 codon, inhA promoter region and rpoB RRDR by MTBDRplus assay and subjected to determination of isoniazid resistance levels by MGIT 960. Discordances were resolved by Sanger sequencing. Among the 203 isolates studied, 109 (54%) were isoniazid-monoresistant, 47 (23%) MDR, 29 (14%) polydrug-resistant, 12 (6%) pre-XDR and 6 (3%) XDR. MTBDRplus detected isoniazid mutations in 75% (153/203) of the isolates. Sequencing of the entire katG and inhA genes revealed mutations in 18/50 wild-type isolates by MTBDRplus (10 with novel mutations), resulting in a total of 32/203 (16%) isolates with no mutations detected. 81/83 (98%) isolates with katG 315 mutations alone had intermediate resistance. Of the 66 isolates with inhA C-15T mutation alone, 51 (77%) showed low-level, 14 (21%) intermediate and 1 (2%) high-level resistance. 5/6 (83%) isolates with mutations in both katG and inhA had high-level resistance. Inferred mutations corresponded to 22% (16/73) of all mutations found in rpoB. Mutations detected in katG regions other than codon 315 in this study might be potential new isoniazid resistance markers and could explain phenotypic resistance in some isolates without katG and inhA classic mutations. In our setting, 16% of isoniazid-resistant isolates, some with high-level resistance, presented no mutations either in katG or inhA.

DOI: 10.1007/s10096-021-04316-0
PMID: 34297229

35. **The economic burden of TB-affected households in DR Congo.**

Int J Tuberc Lung Dis. 2021 Nov 1;25(11):923-932. doi: 10.5588/ijtld.21.0182.

Kaswa M(1), Minga G(2), Nkiere N(2), Mingiedi B(3), Eloko G(4), Nguhiu P(5), Baena IG(6).

BACKGROUND: The Democratic Republic of Congo´s free TB care policy and recent progress with universal health coverage are insufficient to remove barriers to TB care access and adherence. As there were no nationally representative data on the economic burden borne by TB patients, the TB programme conducted a national survey to assess the proportion of TB patients facing catastrophic costs, which could also serve as a baseline for monitoring progress.METHODS: A national survey with retrospective data collection and projection, following WHO methods, was administered to 1,118 patients in 43 treatment zones. Each patient was interviewed once on costs, time loss, coping measures, income, household

expenditure and asset ownership. Total costs were expressed as a percentage of annual household expenditure.RESULTS: In 2019, 56.5% of households affected by TB experienced costs above 20% of their annual household expenditure. Mean costs amounted to respectively US$400 (range: 328-471) and US$1,224 (range: 762-1,686) per episode of first-line and drug-resistant TB. The risk of catastrophic costs increased with hospitalisation, drug resistance status and lower economic status. Half of households resorted to coping strategies and experienced food insecurity. Only 7.5% received social support.CONCLUSION: TB-affected households incur on average a cost of US$549, despite free TB care policy. Mitigating this burden with medical cost reductions, social and labour market measures will be key.

DOI: 10.5588/ijtld.21.0182
PMCID: PMC8544924
PMID: 34686235 [Indexed for MEDLINE]

36. **First report of extensively drug-resistant Mycobacterium tuberculosis (XDR-TB) infection in Kuwait.**

J Infect Public Health. 2021 Nov;14(11):1612-1613. doi: 10.1016/j.jiph.2021.08.020. Epub 2021 Aug 18.

Mokaddas E(1), Ahmad S(2), Eldeen HS(3), Zaglul H(4), Al-Mutairi NM(5), Al-Otaibi A(6).

DOI: 10.1016/j.jiph.2021.08.020
PMID: 34624715

37. **Evaluation of Berberine as an Adjunct to TB Treatment.**

Front Immunol. 2021 Oct 20;12:656419. doi: 10.3389/fimmu.2021.656419. eCollection 2021.

Ozturk M(1)(2), Chia JE(1)(2), Hazra R(3)(4), Saqib M(5), Maine RA(1)(2)(6), Guler R(1)(2)(3), Suzuki H(7), Mishra BB(5), Brombacher F(1)(2)(3), Parihar SP(1)(2)(3)(4).

Tuberculosis (TB) is the global health problem with the second highest number of deaths from a communicable disease after COVID-19. Although TB is curable, poor health infrastructure, long and grueling TB treatments have led to the spread of TB pandemic with alarmingly increasing multidrug-resistant (MDR)-TB prevalence. Alternative host modulating therapies can be employed to improve TB drug

efficacies or dampen the exaggerated inflammatory responses to improve lung function. Here, we investigated the adjunct therapy of natural immune-modulatory compound berberine in C57BL/6 mouse model of pulmonary TB. Berberine treatment did not affect Mtb growth in axenic cultures; however, it showed increased bacterial killing in primary murine bone marrow-derived macrophages and human monocyte-derived macrophages. Ad libitum berberine administration was beneficial to the host in combination with rifampicin and isoniazid. Berberine adjunctive treatment resulted in decreased lung pathology with no additive or synergistic effects on bacterial burdens in mice. Lung immune cell flow cytometry analysis showed that adjunctive berberine treatment decreased neutrophil, CD11b+ dendritic cell and recruited interstitial macrophage numbers. Late onset of adjunctive berberine treatment resulted in a similar phenotype with consistently reduced numbers of neutrophils both in lungs and the spleen. Together, our results suggest that berberine can be supplemented as an immunomodulatory agent depending on the disease stage and inflammatory status of the host.

DOI: 10.3389/fimmu.2021.656419
PMCID: PMC8563784
PMID: 34745081 [Indexed for MEDLINE]

38. **Genomic Profiling of Mycobacterium tuberculosis Strains, Myanmar.**

Emerg Infect Dis. 2021 Nov;27(11):2847-2855. doi: 10.3201/eid2711.210726.

Aung HL, Nyunt WW, Fong Y, Biggs PJ, Winkworth RC, Lockhart PJ, Yeo TW, Hill PC, Cook GM, Aung ST.

Multidrug resistance is a major threat to global elimination of tuberculosis (TB). We performed phenotypic drug-susceptibility testing and whole-genome sequencing for 309 isolates from 342 consecutive patients who were given a diagnosis of TB in Yangon, Myanmar, during July 2016–June 2018. We identified isolates by using the GeneXpert platform to evaluate drug-resistance profiles. A total of 191 (62%) of 309 isolates had rifampin resistance; 168 (88%) of these rifampin-resistant isolates were not genomically related, indicating the repeated emergence of resistance in the population, rather than extensive local transmission. We did not detect resistance mutations to new oral drugs, including bedaquiline and pretomanid. The current GeneXpert MTB/RIF system needs to be modified by using the newly launched Xpert MTB/XDR cartridge or line-probe assay. Introducing new oral drugs to replace those currently used in treatment regimens for multidrug-resistant TB will also be useful for treating TB in

Myanmar.

DOI: 10.3201/eid2711.210726
PMCID: PMC8544997
PMID: 34670644 [Indexed for MEDLINE]

39. **The role of microbiota in respiratory health and diseases, particularly in tuberculosis.**

Biomed Pharmacother. 2021 Nov;143:112108. doi: 10.1016/j.biopha.2021.112108. Epub 2021 Sep 21.

Shah T(1), Shah Z(2), Baloch Z(3), Cui X(4).

Trillions of beneficial and hostile microorganisms live in the human respiratory and gastrointestinal tracts, which act as gatekeepers in maintaining human health, i.e., protecting the body from pathogens by colonizing mucosal surfaces with microbiota-derived antimicrobial metabolites such as short-chain fatty acids or host-derived cytokines and chemokines. It is widely accepted that the microbiome interacts with each other and with the host in a mutually beneficial relationship. Microbiota in the respiratory tract may also play a crucial role in immune homeostasis, maturation, and maintenance of respiratory physiology. Anti-TB antibiotics may cause dysbiosis in the lung and intestinal microbiota, affecting colonization resistance and making the host more susceptible to Mycobacterium tuberculosis (M. tuberculosis) infection. This review discusses recent advances in our understanding of the lung microbiota composition, the lungs and intestinal microbiota related to respiratory health and diseases, microbiome sequencing and analysis, the bloodstream, and the lymphatic system that underpin the gut-lung axis in M. tuberculosis-infected humans and animals. We also discuss the gut-lung axis interactions with the immune system, the role of the microbiome in TB pathogenesis, and the impact of anti-TB antibiotic therapy on the microbiota in animals, humans, and drug-resistant TB individuals.

DOI: 10.1016/j.biopha.2021.112108
PMID: 34560539

40. **Profiling and identification of novel rpoB mutations in rifampicin-resistant Mycobacterium tuberculosis clinical isolates from Pakistan.**

J Infect Chemother. 2021 Nov;27(11):1578-1583. doi: 10.1016/j.jiac.2021.06.020.

Epub 2021 Jul 7.

Qadir M(1), Tahseen S(2), McHugh TD(3), Hussain A(2), Masood F(2), Ahmed N(2), Faryal R(4).

INTRODUCTION: Rifampicin (RIF) is one of the most effective anti-tuberculosis first-line drugs prescribed along with isoniazid. However, the emergence of RIF resistance Mycobacterium tuberculosis (MTB) isolates is a major issue towards tuberculosis (TB) control program in high MDR TB-burdened countries including Pakistan. Molecular data behind phenotypic resistance is essential for better management of RIF resistance which has been linked with mutations in rpoB gene. Since molecular studies on RIF resistance is limited in Pakistan, the current study was aimed to investigate the molecular data of mutations in rpoB gene behind phenotypic RIF resistance isolates in Pakistan.
METHOD: A total of 322 phenotypically RIF-resistant isolates were randomly selected from National TB Reference Laboratory, Pakistan for sequencing while 380 RIF resistance whole-genome sequencing (WGS) of Pakistani isolates (BioProject PRJEB25972), were also analyzed for rpoB mutations.
RESULT: Among the 702 RIF resistance samples, 675 (96.1%) isolates harbored mutations in rpoB in which 663 (94.4%) were detected within the Rifampicin Resistance Determining Region (RRDR) also known as a mutation hot spot region, including three novel. Among these mutations, 657 (97.3%) were substitutions including 603 (89.3%) single nucleotide polymorphism, 49 (7.25%) double and five (0.8%) triple. About 94.4% of Phenotypic RIF resistance strains, exhibited mutations in RRDR, which were also detectable by GeneXpert.
CONCLUSION: Mutations in the RRDR region of rpoB is a major mechanism of RIF resistance in MTB circulating isolates in Pakistan. Molecular detection of drug resistance is a faster and better approach than phenotypic drug susceptibility testing to reduce the time for transmission of RIF resistance strains in population. Such insights will inform the deployment of anti-TB drug regimens and disease control tools and strategies in high burden settings, such as Pakistan.

DOI: 10.1016/j.jiac.2021.06.020
PMID: 34244055 [Indexed for MEDLINE]

41. **Barriers and strategies to successful tuberculosis treatment in a high-burden tuberculosis setting: a qualitative study from the patient's perspective.**

BMC Public Health. 2021 Oct 21;21(1):1903. doi: 10.1186/s12889-021-12005-y.

Pradipta IS(1)(2)(3), Idrus LR(4)(5), Probandari A(6)(7), Lestari BW(8)(9), Diantini A(10), Alffenaar JC(11)(12)(13), Hak E(4).

BACKGROUND: Previously treated tuberculosis (TB) patients are a widely reported risk factor for multidrug-resistant tuberculosis. Identifying patients' problems during treatment is necessary to control TB, especially in a high-burden setting. We therefore explored barriers to successful TB treatment from the patients' perspective, aiming to identify potential patient-centred care strategies to improve TB treatment outcome in Indonesia.
METHODS: A qualitative study was conducted in a province of Indonesia with high TB prevalence. Participants from various backgrounds (i.e., TB patients, physicians, nurses, pharmacists, TB activist, TB programmers at the district and primary care levels) were subject to in-depth interviews and focus group discussions (FGDs). All interviews and FGDs were transcribed verbatim from audio and visual recordings and the respective transcriptions were used for data analysis. Barriers were constructed by interpreting the codes' pattern and co-occurrence. The information's trustworthiness and credibility were established using information saturation, participant validation and triangulation approaches. Data were inductively analysed using the Atlas.ti 8.4 software and reported following the COREQ 32-items.
RESULTS: We interviewed 63 of the 66 pre-defined participants and identified 15 barriers. The barriers were classified into three themes, i.e., socio-demography and economy; knowledge and perception and TB treatment. Since the barriers can be interrelated, we determined five main barriers across all barrier themes, i.e., lack of TB knowledge, stigmatisation, long distance to the health facility, adverse drug reaction and loss of household income.
CONCLUSION: The main treatment barriers can be considered to strengthen patient-centred care for TB patients in Indonesia. A multi-component approach including TB patients, healthcare providers, broad community and policy makers is required to improve TB treatment success.

DOI: 10.1186/s12889-021-12005-y
PMCID: PMC8529853
PMID: 34670527 [Indexed for MEDLINE]

42. **Isoniazid-Loaded Albumin Nanoparticles: Taguchi Optimization Method.**

Polymers (Basel). 2021 Nov 4;13(21):3808. doi: 10.3390/polym13213808.

Tazhbayev Y(1), Galiyeva A(1), Zhumagaliyeva T(1), Burkeyev M(1), Karimova B(1).

Tuberculosis is one of the dangerous infectious diseases, killing over a million people worldwide each year. The search for new dosage forms for the treatment of drug-resistant tuberculosis is an actual task. Biocompatible polymer nanoparticles, in particular bovine serum albumin (BSA), are promising drug carriers. Nanoparticle (NP) parameters such as diameter, polydispersity, bioactive substance loading, and NP yield are very important when it comes to drug transport through the bloodstream. The most well-known and widely used first-line anti-tuberculosis drug, isoniazid (INH), is being used as a drug. BSA-INH NPs were obtained by an ethanol desolvation of an aqueous protein solution in the drug presence. The peculiarity of the method is that natural components, namely urea and cysteine, are used for the stabilization of BSA-INH NPs after desolvation. The characteristics of the obtained BSA-INH NPs are significantly affected by the concentration of protein, isoniazid, urea, and cysteine in the solution. The aim of the present study is to investigate the concentration effect of the system reacting components on the parameters of the NPs that are obtained. We have chosen the concentrations of four reacting components, i.e., BSA, isoniazid, urea, and cysteine, as controlling factors and applied the Taguchi method to analyze which concentration of each component has an important effect on BSA-INH NPs characteristics.

DOI: 10.3390/polym13213808
PMCID: PMC8588201
PMID: 34771365

43. **Revised nomenclature and SNP barcode for Mycobacterium tuberculosis lineage 2.**

Microb Genom. 2021 Nov;7(11). doi: 10.1099/mgen.0.000697.

Thawornwattana Y(1)(2), Mahasirimongkol S(3), Yanai H(4), Maung HMW(5)(6), Cui Z(6)(7), Chongsuvivatwong V(6), Palittapongarnpim P(1)(8).

Mycobacterium tuberculosis (Mtb) lineage 2 (L2) strains are present globally, contributing to a widespread tuberculosis (TB) burden, particularly in Asia where both prevalence of TB and numbers of drug resistant TB are highest. The increasing availability of whole-genome sequencing (WGS) data worldwide provides an opportunity to improve our understanding of the global genetic diversity of Mtb L2 and its association with the disease epidemiology and pathogenesis. However, existing L2 sublineage classification schemes leave >20% of the Modern Beijing isolates unclassified. Here, we present a revised SNP-based classification scheme of L2 in a genomic framework based on phylogenetic analysis of >4000 L2 isolates from 34 countries in Asia, Eastern Europe, Oceania and Africa. Our scheme consists of over 30 genotypes, many of which have not been described before. In particular, we propose six main genotypes of Modern

Beijing strains, denoted L2.2.M1-L2.2.M6. We also provide SNP markers for genotyping L2 strains from WGS data. This fine-scale genotyping scheme, which can classify >98% of the studied isolates, serves as a basis for more effective monitoring and reporting of transmission and outbreaks, as well as improving genotype-phenotype associations such as disease severity and drug resistance. This article contains data hosted by Microreact.

DOI: 10.1099/mgen.0.000697
PMID: 34787541

44. **Utility of EBUS-TBNA in diagnosing mediastinal tuberculous lymphadenitis in East London.**

J Infect. 2021 Oct 24:S0163-4453(21)00531-4. doi: 10.1016/j.jinf.2021.10.015. Online ahead of print.

Lucey O(1), Potter J(2), Ricketts W(2), Castle L(2), Melzer M(3).

OBJECTIVES: To characterise and describe the diagnostic utility of Endobronchial ultrasound-transbronchial needle aspiration (EBUS-TBNA) in intrathoracic tuberculosis in a cohort of patients with mediastinal lymphadenopathy of unknown aetiology.
METHODS: Consecutive patients with intrathoracic lymphadenopathy undergoing EBUS-TBNA between 2012 and 2016 were identified. Demographic data, biopsy cytopathology and mycobacteriology results, HIV and vitamin D status, susceptibility results and final diagnoses were recorded. Pre- and post-procedure probability scores were assigned to each case to reflect the probability of tuberculosis.
RESULTS: 315 cases were identified; 54 (17.1%) had tuberculosis and 261 (82.9%) had a non-tuberculosis diagnosis. amongst TB cases, the sensitivity of EBUS-TBNA was 59.3% (95% CI 45.06-72.14), specificity 100% (95% CI 98.19-100) and the negative predictive value (NPV) was 92.23% (95% CI 88.31-94.95). 19/54 (35%) TB cases were confirmed by EBUS mycobacterial culture and 13/54 (24.1%) by cytopathology. 33 (61.1%) of the TB cases, had a low to medium pre-test probability score assigned prior to EBUS-TBNA. Amongst EBUS culture-confirmed cases, we found a resistance rate of 10.5% to one or more first line TB drugs, with one case of multi-drug resistant TB.
CONCLUSIONS: We confirmed the utility of EBUS-TBNA in the diagnosis of intrathoracic tuberculosis in an undifferentiated cohort of patients with mediastinal lymphadenopathy of unknown aetiology and advocate sending samples for mycobacterial culture in all cases in high tuberculosis incidence areas.

DOI: 10.1016/j.jinf.2021.10.015
PMID: 34706281

45. **Tuberculosis infection control measures in healthcare facilities in Moyen-Ogooué Province, Gabon.**

BMC Health Serv Res. 2021 Nov 5;21(1):1200. doi: 10.1186/s12913-021-07236-z.

Vigenschow A(#)(1)(2), Adegbite BR(#)(1)(2)(3), Edoa JR(1)(2), Alabi A(1), Adegnika AA(1)(2), Grobusch MP(4)(5)(6)(7)(8), Massinga-Loembe M(1)(2).

BACKGROUND: Healthcare workers (HCW) are at higher risk of tuberculosis (TB) than the general population. We assessed healthcare facilities for their TB infection control standards and priorities.
METHODS: A standardised tool was applied. The assessment was conducted by direct observation, documents review and interviews with the facility heads.
RESULTS: Twenty healthcare facilities were assessed; 17 dispensaries, an HIV-clinic, a private not-for-profit hospital and a public regional hospital. In both hospitals, outpatient departments, internal medicine wards, paediatric wards, emergency departments; and the MDR-TB unit of the public regional hospital were assessed. In Gabon, there are currently no national guidelines for TB infection control (TBIC) in healthcare settings. Consequently, none of the facilities had an infection control plan or TBIC focal point. In three departments of two facilities (2/20 facilities), TB patients and presumed TB cases were observed to be consistently provided with surgical masks. One structure reported to regularly test some of its personnel for TB. Consultation rooms were adequately ventilated in six primary care level facilities (6/17 dispensaries) and in none of the hospitals, due to the use of air conditioning. Adequate personal protective equipment was not provided regularly by the facilities and was only found to be supplied in the MDR-TB unit and one of the paediatric wards.
CONCLUSIONS: In Moyen-Ogooué province, implementation of TBIC in healthcare settings is generally low. Consequently, HCW are not sufficiently protected and therefore at risk for M. tuberculosis infection. There is an urgent need for national TBIC guidelines and training of health workers to safeguard implementation.

DOI: 10.1186/s12913-021-07236-z
PMCID: PMC8571857

PMID: 34740361 [Indexed for MEDLINE]

46. **Resurgence of tuberculosis amid COVID-19 in Peru: Associated risk factors and recommendations.**

Int J Health Plann Manage. 2021 Nov;36(6):2441-2445. doi: 10.1002/hpm.3291. Epub 2021 Jul 27.

Khan FMA(1), Kazmi Z(2), Hasan MM(3)(4), Dos Santos Costa AC(5), Ahmad S(6), Essar MY(7).

Peru is one of the countries with the highest incidence of tuberculosis and multidrug-resistant tuberculosis in the world. Although public health measures adopted in the country have improved the care, diagnosis and management of patients with tuberculosis, there are still failures in the control of the disease in the country, especially of multidrug-resistant tuberculosis and among the prison population or people living with HIV. The COVID-19 pandemic has added a great burden to the Peruvian public health system, negatively impacting tuberculosis-focused health programs due to the diversion of resources to control the pandemic. Consequently, combat measures, epidemiological surveillance of tuberculosis cases were affected, and data point to an increase in the number of cases, especially of multidrug-resistant tuberculosis, and to the underdiagnosis of the disease. To deal with this problem and avoid a future catastrophe for the country's health system, multidisciplinary measures involving the population, health professionals and government bodies are needed. It is essential that education, diagnosis, contact screening and treatment programs are prioritised and given greater financial support. Furthermore, it is necessary to raise awareness in the population about the need for isolation and maintenance of treatment, especially among the most vulnerable populations.

DOI: 10.1002/hpm.3291
PMID: 34318523 [Indexed for MEDLINE]

47. **Characterisation of a putative M23-domain containing protein in Mycobacterium tuberculosis.**

PLoS One. 2021 Nov 16;16(11):e0259181. doi: 10.1371/journal.pone.0259181. eCollection 2021.

Papadopoulos AO(1), Ealand C(1), Gordhan BG(1), VanNieuwenhze M(2), Kana BD(1).

Mycobacterium tuberculosis, the causative agent of tuberculosis remains a global health concern, further compounded by the high rates of HIV-TB co-infection and emergence of multi- and extensive drug resistant TB, all of which have hampered efforts to eradicate this disease. As a result, novel anti-tubercular interventions are urgently required, with the peptidoglycan component of the M. tuberculosis cell wall emerging as an attractive drug target. Peptidoglycan M23 endopeptidases can function as active cell wall hydrolases or degenerate activators of hydrolases in a variety of bacteria, contributing to important processes such as bacterial growth, division and virulence. Herein, we investigate the function of the Rv0950-encoded putative M23 endopeptidase in M. tuberculosis. In silico analysis revealed that this protein is conserved in mycobacteria, with a zinc-binding catalytic site predictive of hydrolytic activity. Transcript analysis indicated that expression of Rv0950c was elevated during lag and log phases of growth and reduced in stationary phase. Deletion of Rv0950c yielded no defects in growth, colony morphology, antibiotic susceptibility or intracellular survival but caused a reduction in cell length. Staining with a monopeptide-derived fluorescent D-amino acid, which spatially reports on sites of active PG biosynthesis or repair, revealed an overall reduction in uptake of the probe in ΔRv0950c. When stained with a dipeptide probe in the presence of cell wall damaging agents, the ΔRv0950c mutant displayed reduced sidewall labelling. As bacterial peptidoglycan metabolism is important for survival and pathogenesis, the role of Rv0950c and other putative M23 endopeptidases in M. tuberculosis should be explored further.

DOI: 10.1371/journal.pone.0259181
PMCID: PMC8594824
PMID: 34784363

48. **Intracellular Accumulation of Novel and Clinically Used TB Drugs Potentiates Intracellular Synergy.**

Microbiol Spectr. 2021 Oct 31;9(2):e0043421. doi: 10.1128/Spectrum.00434-21. Epub 2021 Sep 29.

Tanner L(1), Mashabela GT(2), Omollo CC(2), de Wet TJ(2), Parkinson CJ(3), Warner DF(2)(4), Haynes RK(5), Wiesner L(1).

The therapeutic repertoire for tuberculosis (TB) remains limited despite the existence of many TB drugs that are highly active in in vitro models and possess clinical utility. Underlying the lack of efficacy in vivo is the inability of TB drugs to penetrate microenvironments inhabited by the causative agent, Mycobacterium tuberculosis, including host alveolar macrophages. Here, we

determined the ability of the phenoxazine PhX1 previously shown to be active against M. tuberculosis in vitro to differentially penetrate murine compartments, including plasma, epithelial lining fluid, and isolated epithelial lining fluid cells. We also investigated the extent of permeation into uninfected and M. tuberculosis-infected human macrophage-like Tamm-Horsfall protein 1 (THP-1) cells directly and by comparing to results obtained in vitro in synergy assays. Our data indicate that PhX1 (4,750 ± 127.2 ng/ml) penetrates more effectively into THP-1 cells than do the clinically used anti-TB agents, rifampin (3,050 ± 62.9 ng/ml), moxifloxacin (3,374 ± 48.7 ng/ml), bedaquiline (4,410 ± 190.9 ng/ml), and linezolid (770 ± 14.1 ng/ml). Compound efficacy in infected cells correlated with intracellular accumulation, reinforcing the perceived importance of intracellular penetration as a key drug property. Moreover, we detected synergies deriving from redox-stimulatory combinations of PhX1 or clofazimine with the novel prenylated amino-artemisinin WHN296. Finally, we used compound synergies to elucidate the relationship between compound intracellular accumulation and efficacy, with PhX1/WHN296 synergy levels shown to predict drug efficacy. Collectively, our data support the utility of the applied assays in identifying in vitro active compounds with the potential for clinical development. IMPORTANCE This study addresses the development of novel therapeutic compounds for the eventual treatment of drug-resistant tuberculosis. Tuberculosis continues to progress, with cases of Mycobacterium tuberculosis (M. tuberculosis) resistance to first-line medications increasing. We assess new combinations of drugs with both oxidant and redox properties coupled with a third partner drug, with the focus here being on the potentiation of M. tuberculosis-active combinations of compounds in the intracellular macrophage environment. Thus, we determined the ability of the phenoxazine PhX1, previously shown to be active against M. tuberculosis in vitro, to differentially penetrate murine compartments, including plasma, epithelial lining fluid, and isolated epithelial lining fluid cells. In addition, the extent of permeation into human macrophage-like THP-1 cells and H37Rv-infected THP-1 cells was measured via mass spectrometry and compared to in vitro two-dimensional synergy and subsequent intracellular efficacy. Collectively, our data indicate that development of new drugs will be facilitated using the methods described herein.

DOI: 10.1128/Spectrum.00434-21
PMCID: PMC8557888
PMID: 34585951

49. **Immunotherapeutic effect of adenovirus encoding antimicrobial peptides in experimental pulmonary tuberculosis.**

J Leukoc Biol. 2021 Nov;110(5):951-963. doi: 10.1002/JLB.4MA0920-627R. Epub 2021 Mar 8.

Ramos-Espinosa O(1), Mata-Espinosa D(1), Francisco-Cruz A(2), López-Torres MO(1), Hernández-Bazán S(1), Barrios-Payán J(1), Marquina-Castillo B(1), Carretero M(3)(4), Del Río M(3)(4), Hernández-Pando R(1).

As components of the innate immune response, antimicrobial peptides (AMPs) efficiently contribute to infection control and maintenance of a latent state in pulmonary tuberculosis (TB). As a therapeutic strategy, the administration of recombinant AMPs could be limited by enzymatic degradation and high production costs. Likewise, strategies based on the induction of AMPs have generated controversial results. In this study, 2 recombinant type-5 adenoviruses (Ad) expressing the human β-defensin 3 (HβD3) or cathelicidin (LL37) were assessed in a murine pulmonary TB model. Mice infected with either a high dose of a drug-sensitive (H37Rv) or a multidrug-resistant (MDR) strain of Mycobacterium tuberculosis (Mtb) were treated with a single administration of AdHβD3, AdLL37, AdGFP (control vector expressing a green fluorescent protein), or saline solution (SS). Lungs were obtained to determine the bacterial burden, histologic damage, and cytokine expression at different time points. Mice treated with AdHβD3 or AdLL37 showed significantly lower bacterial load and pneumonia, and higher proinflammatory cytokine expression than the control groups AdGFP and SS. A synergistic therapeutic effect could be observed when first- or second-line antibiotics (ABs) were administered with adenoviral therapy in animals infected with H37Rv or MDR strains, respectively. Adenovirus-delivered AMP's administration constitutes a promising adjuvant therapy for current anti-TB drugs by enhancing a protective immune response and potentially reducing current AB regimes' duration.

DOI: 10.1002/JLB.4MA0920-627R
PMID: 33682193 [Indexed for MEDLINE]

50. **Discovery of 5-methylpyrimidopyridone analogues as selective antimycobacterial agents.**

Bioorg Med Chem. 2021 Nov 1;49:116426. doi: 10.1016/j.bmc.2021.116426. Epub 2021 Sep 27.

Wu Y(1), Cheung CY(2), Zhou Y(1), Wang Z(1), Tu Z(1), Cook GM(3), Lu X(4).

With the emergence of multidrug-resistant strains of Mycobacterium tuberculosis (MDR-TB) and extensive drug-resistant strains (XDR-TB), there is an urgent need to develop novel drugs for the treatment of tuberculosis. Here, we designed and

synthesized a series of 5-methylpyrimidopyridone analogues as potential antitubercular agents. The most potent compound 6q exhibited a MIC value of 4 μM in vitro against Mycobacterium tuberculosis. The antitubercular activities of the synthesized compounds were impacted by the amantadine and 2-chlorophenyl groups, and were enhanced by the presence of 3-methyl(4-dimethylamino)piperidinylphenyl. Molecular modeling and binding studies suggest that PknB is the potential molecular target of 5-methylpyrimidopyridone compounds. This study provides insights for the future development of new antimycobacterial agents with novel mechanisms of action.

DOI: 10.1016/j.bmc.2021.116426
PMID: 34624820

51. **Safety and Effectiveness of an All-Oral, Bedaquiline-Based, Shorter Treatment Regimen for Rifampicin-Resistant Tuberculosis in High Human Immunodeficiency Virus (HIV) Burden Rural South Africa: A Retrospective Cohort Analysis.**

Clin Infect Dis. 2021 Nov 2;73(9):e3563-e3571. doi: 10.1093/cid/ciaa1894.

Tack I(1), Dumicho A(2), Ohler L(1), Shigayeva A(2), Bulti AB(1), White K(1), Mbatha M(3), Furin J(4), Isaakidis P(5).

BACKGROUND: At the end of 2018, South Africa updated its all-oral regimen, to include bedaquiline (BDQ) and 2 months of linezolid (LZD) for all patients initiating the shorter 9-12 months regimen for rifampicin-resistant tuberculosis (RR-TB). We assessed a group of patients in rural KwaZulu-Natal for safety and effectiveness of this treatment regimen under programmatic conditions.
METHODS: We conducted a retrospective cohort analysis on RR-TB patients treated with a standardized all-oral short regimen between 1 July 2018 and 30 April 2019 in 3 facilities in King Cetshwayo District. An electronic register (EDR web) and facility-based clinical charts were used to collect variables, which were entered into an Epi-Info database.
RESULTS: Our cohort included 117 patients; 68.4% (95% confidence interval [CI]: 59.3-76.3) tested positive for human immunodeficiency virus (HIV). The median time to culture conversion was 56 days (95% CI: 50-57). Treatment success was achieved in 75.2% (95% CI: 66.5-82.3) of patients. Mortality within the cohort was 12.8% (95% CI: 7.8-20.3). Anemia was the most frequent severe adverse event (AE). The median time to develop severe anemia was 7.1 weeks (interquartile range [IQR] 4.0-12.9) after treatment initiation. LZD was interrupted in 25.2% (95% CI: 17.8-34.5) of participants.
CONCLUSIONS: An all-oral shorter regimen, including BDQ and LZD as core drugs

for the treatment of RR-TB, shows good outcomes, in a high HIV burden rural setting. AEs are common, especially for LZD, but could be managed in the program setting. Support is needed when introducing new regimens to train staff in the monitoring, management, and reporting of AEs.

DOI: 10.1093/cid/ciaa1894
PMCID: PMC8563184
PMID: 33372989 [Indexed for MEDLINE]

52. **IMB-XMA0038, a new inhibitor targeting aspartate-semialdehyde dehydrogenase of Mycobacterium tuberculosis.**

Emerg Microbes Infect. 2021 Nov 15:1-28. doi: 10.1080/22221751.2021.2006578. Online ahead of print.

Wang X(1), Yang R(2), Liu S(1), Guan Y(1), Xiao C(1), Li C(2), Meng J(1), Pang Y(2), Liu Y(1).

AbstractThe emergence of drug-resistant tuberculosis (TB) constitutes a major challenge to TB control programs. There is an urgent need to develop effective anti-TB drugs with novel mechanisms of action. Aspartate-semialdehyde dehydrogenase (ASADH) is the second enzyme in the aspartate metabolic pathway. The absence of the pathway in humans and the absolute requirement of aspartate in bacteria make ASADH a highly attractive drug target. In this study, we used ASADH coupled with Escherichia coli type III aspartate kinase (LysC) to establish a high-throughput screening method to find new anti-TB inhibitors. IMB-XMA0038 was identified as an inhibitor of MtASADH with an IC50 value of 0.59 μg/mL through screening. The interaction between IMB-XMA0038 and MtASADH was confirmed by surface plasmon resonance (SPR) assay and molecular docking analysis. Furthermore, IMB-XMA0038 was found to inhibit various drug-resistant MTB strains potently with minimal inhibitory concentrations (MICs) of 0.25-0.5 μg/mL. The conditional mutant strain MTB::asadh cultured with different concentrations of inducer (10-5 or 10-1 μg/mL pristinamycin) resulted in a maximal 16 times difference in MICs. At the same time, IMB-XMA0038 showed low cytotoxicity in vitro and vivo. In mouse model, it encouragingly declined the MTB colony forming units (CFU) in lung by 1.67 log10 dosed at 25 mg/kg for 15 days. In conclusion, our data demonstrate that IMB-XMA0038 is a promising lead compound against drug-resistant tuberculosis.

DOI: 10.1080/22221751.2021.2006578

PMID: 34779708

53. **Host factors subverted by Mycobacterium tuberculosis: Potential targets for host directed therapy.**

Int Rev Immunol. 2021 Oct 22:1-28. doi: 10.1080/08830185.2021.1990277. Online ahead of print.

Kalra R(1), Tiwari D(1), Dkhar HK(1), Bhagyaraj E(1), Kumar R(2)(3), Bhardwaj A(2)(3), Gupta P(1)(3).

INTRODUCTION: Despite new approaches in the diagnosis and treatment of tuberculosis (TB), it continues to be a major health burden. Several immunotherapies that potentiate the immune response have come up as adjuncts to drug therapies against drug resistant TB strains; however, there needs to be an urgent appraisal of host specific drug targets for improving their clinical management and to curtail disease progression. Presently, various host directed therapies (HDTs) exist (repurposed drugs, nutraceuticals, monoclonal antibodies and immunomodulatory agents), but these mostly address molecules that combat disease progression.
AREAS COVERED: The current review discusses major Mycobacterium tuberculosis (M. tuberculosis) survival paradigms inside the host and presents a plethora of host targets subverted by M. tuberculosis which can be further explored for future HDTs. The host factors unique to M. tuberculosis infection have also been identified through an in-silico interaction mapping.
EXPERT OPINION: HDTs could become the next-generation adjunct therapies in order to counter antimicrobial resistance and virulence, as well as to reduce the duration of existing TB treatments. However, current scientific efforts are largely directed toward combatants rather than host molecules co-opted by M. tuberculosis for its survival. This might drive the immune system to a hyper-inflammatory condition; therefore, we emphasize that host factors subverted by M. tuberculosis, and their subsequent neutralization, must be considered for development of better HDTs.

DOI: 10.1080/08830185.2021.1990277
PMID: 34678117

54. **Safety, Tolerability, and Pharmacokinetics of Telacebec (Q203), a New Antituberculosis Agent, in Healthy Subjects.**

Antimicrob Agents Chemother. 2021 Oct 25:AAC0143621. doi: 10.1128/AAC.01436-21. Online ahead of print.

Kim J(1)(2), Choi J(2), Kang H(2), Ahn J(2), Hutchings J(2), van Niekerk C(2), Park D(2), Kim J(2), Jeon Y(2), Nam K(2), Shin S(3), Shin BS(1).

Telacebec (Q203) is a potent drug candidate under clinical development for the treatment of drug-naïve and drug-resistant tuberculosis. The first-in-human randomized, placebo-controlled, double-blind, dose-escalation Phase 1A trial (Q203-TB-PI-US001) was conducted to evaluate the safety, tolerability, and pharmacokinetics of telacebec. A total of 56 normal, healthy, male and female subjects (42 active and 14 placebo) were enrolled in the study. The doses of telacebec were 10 mg (Cohort 1), 30 mg (Cohort 2), 50 mg (Cohort 3), 100 mg (Cohort 4), 200 mg (Cohort 5), 400 mg (Cohort 6), and 800 mg (Cohort 7) in a fasted state. Subjects participating in Cohort 4 were also enrolled in Cohort 8 to investigate the food effect on the pharmacokinetics of telacebec after a high-fat meal. In all subjects dosed with telacebec (10 - 800 mg), telacebec was well tolerated and did not lead to any significant or serious adverse events. Following a single oral administration of telacebec (10 - 800 mg), telacebec plasma concentration reached the maximal plasma concentration (Cmax) in average 2.0 - 3.5 h and showed multi-exponential decline thereafter. The area under the plasma concentration vs. time curve (AUC) was approximately dose-proportional. A significant increase in plasma concentrations was observed in the fed condition compared with the fasted condition with the geometric mean ratio of 3.93 for Cmax. Moderate delay in Tmax (4.5 h) was also observed in the fed condition. These results, combined with the demonstrated activity against drug-sensitive and multidrug-resistant Mycobacterium tuberculosis, support further investigation of telacebec for the treatment of tuberculosis.

DOI: 10.1128/AAC.01436-21
PMID: 34694872

55. **Community-Based Ototoxicity Monitoring for Drug-Resistant Tuberculosis in South Africa: An Evaluation Study.**

Int J Environ Res Public Health. 2021 Oct 28;18(21):11342. doi: 10.3390/ijerph182111342.

Stevenson LJ(1), Biagio-de Jager L(1), Graham MA(2), Swanepoel W(1)(3).

In response to the drug-resistant tuberculosis (DRTB) ototoxicity burden in South Africa, ototoxicity monitoring has been decentralised, with community health workers (CHWs) acting as facilitators. This study describes a community-based ototoxicity monitoring programme (OMP) for patients with DRTB. Findings are compared to the recommended guidelines for ototoxicity monitoring,

the OMP protocol and published studies. This was a retrospective study of longitudinal ototoxicity monitoring of 831 patients with DRTB, using data collected at community-based clinics in the City of Cape Town between 2013 and 2017. Approximately half (46.8%) of the patients had an initial assessment conducted in accordance with the OMP protocol recommendations, and follow-up rates (79.5%) were higher than those of a similar DRTB programme. However, patients in this study were not monitored within the timeframes or with the regularity recommended by the guidelines or the OMP protocol. Extended high-frequency pure-tone audiometry (27.5%) was underutilised by testers and data recording was inconsistent (e.g., 37.7% of patient gender was not recorded by testers). Community-based OMP using CHWs to facilitate monitoring showed improvement over previous hospital-based reports, with more accessible services and higher follow-up rates. However, to improve OMP outcomes, OMP managers should reassess current protocols and data recording practices.

DOI: 10.3390/ijerph182111342
PMCID: PMC8583517
PMID: 34769860

56. **Gauging the impact of the COVID-19 pandemic on tuberculosis services: a global study.**

Eur Respir J. 2021 Nov 11;58(5):2101786. doi: 10.1183/13993003.01786-2021. Print 2021 Nov.

Migliori GB(1), Thong PM(2), Alffenaar JW(3)(4)(5), Denholm J(6)(7), Tadolini M(8)(9), Alyaquobi F(10), Blanc FX(11), Buonsenso D(12), Cho JG(4)(5)(13), Codecasa LR(14), Danila E(15), Duarte R(16), García-García JM(17), Gualano G(18), Rendon A(19), Silva DR(20), Souleymane MB(21), Tham SM(22), Thomas TA(23), Tiberi S(24)(25), Udwadia ZF(26), Goletti D(18), Centis R(1), D'Ambrosio L(27), Sotgiu G(28), Ong CWM(29)(22)(30); Global Tuberculosis Network.

This global study of 43 TB centres from 19 countries demonstrates the impact of COVID-19 pandemic on TB services. Newly diagnosed TB disease, drug-resistant TB, TB deaths, outpatient clinic attendances and newly diagnosed TB infection were reduced. https://bit.ly/3sdHbfk

The effects of the coronavirus disease 2019 (COVID-19) pandemic on tuberculosis (TB) disease and TB services emerged in the beginning of 2020 [1, 2]. Epidemiological and clinical studies, including mortality rates of the first cohort of patients with COVID-19 and TB co-infection were described [3, 4]. Several reports from individual countries suggested that the COVID-19 pandemic significantly affected TB services [5–9], including validation by modelling

studies [10]. The Global Tuberculosis Network (GTN) reported that the COVID-19 pandemic affected TB services in 33 TB centres from 16 countries in the first 4 months of 2020 [11]. An increased use of telehealth during the COVID-19 pandemic was observed in some TB centres [11]. The major limitations of that study were the short period of observation (January to April 2020 compared to the same period in 2019) and the limited number of variables analysed [11–14].

DOI: 10.1183/13993003.01786-2021
PMCID: PMC8581650
PMID: 34446465 [Indexed for MEDLINE]

57. **Prolonged use of bedaquiline in the treatment for MDR-TB in a child.**

IDCases. 2021 Oct 20;26:e01311. doi: 10.1016/j.idcr.2021.e01311. eCollection 2021.

Gubkina MF(1), Khokhlova JY(1), Yukhimenko NV(1), Petrakova IY(1).

Bedaquiline (Bdq), a novel TB drug, is referred to the most effective drugs and used for the management of multidrug-resistant tuberculosis (MDR-TB). The drug produces a cardiotoxic effect, and its use is limited to six months. We describe a clinical observation of prolonged bedaquiline use in the treatment for MDR-TB using a restricted number of drugs, to which susceptibility was preserved, in a 12-year-old child. The previous treatment course had failed; the patient remained sputum-positive after eight months of the treatment. We used a personalized approach to chemotherapy correction based on repeat drug susceptibility testing. The treatment regimen only contained those drugs, to which susceptibility was preserved: amikacin, cycloserine, linezolid, bedaquiline (AmCsLzdBdq). Amikacin was withdrawn after three months due to the development of sensorineural hearing loss. The treatment was continued with CsLzdBdq. The total chemotherapy course took 18 months. Sputum conversion was observed after one month, cavity closure - by 18 months of treatment. We did not observe cardiotoxic effects due to prolonged bedaquiline use. The administration of prolonged bedaquiline use was based on life-saving considerations. We achieved favourable treatment outcome and demonstrated safety of prolonged bedaquiline use for a child.

DOI: 10.1016/j.idcr.2021.e01311
PMCID: PMC8551576
PMID: 34745886

58. **1H-1,2,3-triazole embedded Isatin-Benzaldehyde-bis(heteronuclearhydrazones): Design, Synthesis, anti-mycobacterial, and Cytotoxic evaluation.**

Chem Biol Drug Des. 2021 Nov 16. doi: 10.1111/cbdd.13984. Online ahead of print.

Sharma B(1), Kumar S(1), Preeti(1), Johansen MD(2)(3), Kremer L(2)(4), Kumar V(1).

Rapid growth of global drug-resistant tuberculosis and urgent requirement for short treatment regimens is stimulating the need for discovery of new TB drugs. In this work, we report the design, synthesis and in vitro anti-mycobacterial evaluation of a library of isatin-derived bis(heteronuclear hydrazones). Evaluation results revealed that the inclusion of isoniazid core into 1H-1,2,3-triazole tethered isatin-benzaldehydes improved the anti-mycobacterial activity on tuberculosis mc2 6230 strain and significantly reduced the cytotoxicity against Vero cells. However, the introduction of semicarbazones/thiosemicarbazones or pyrazine-2-carbohydrazide produced the opposite effects. The compounds with isoniazid and polar-donating groups at C-5 position of isatin emerged as the most promising conjugates with MIC99 = 0.36 μg/mL. The most active compounds were non-cytotoxic to Vero cells (IC50 >100 μg/mL) with a selectivity index >277.

DOI: 10.1111/cbdd.13984
PMID: 34786862

59. **In Vitro Activity of Bedaquiline and Imipenem against Actively Growing, Nutrient-Starved, and Intracellular Mycobacterium abscessus.**

Antimicrob Agents Chemother. 2021 Nov 17;65(12):e0154521. doi: 10.1128/AAC.01545-21. Epub 2021 Sep 13.

Martins O(1), Lee J(1), Kaushik A(1), Ammerman NC(1)(2), Dooley KE(1), Nuermberger EL(1).

Mycobacterium abscessus lung disease is difficult to treat due to intrinsic drug resistance and the persistence of drug-tolerant bacteria. Currently, the standard of care is a multidrug regimen with at least 3 active drugs, preferably including a β-lactam (imipenem or cefoxitin). These regimens are lengthy and toxic and have limited efficacy. The search for more efficacious regimens led us to evaluate bedaquiline, a diarylquinoline licensed for treatment of

multidrug-resistant tuberculosis. We performed in vitro time-kill experiments to evaluate the activity of bedaquiline alone and in combination with the first-line drug imipenem against M. abscessus under various conditions. Against actively growing bacteria, bedaquiline was largely bacteriostatic and antagonized the bactericidal activity of imipenem. Contrarily, against nutrient-starved persisters, bedaquiline was bactericidal, while imipenem was not, and bedaquiline drove the activity of the combination. In an intracellular infection model, bedaquiline and imipenem had additive bactericidal effects. Correlations between ATP levels and the bactericidal activity of imipenem and its antagonism by bedaquiline were observed. Interestingly, the presence of Tween 80 in the media affected the activity of both drugs, enhancing the activity of imipenem and reducing that of bedaquiline. Overall, these results show that bedaquiline and imipenem interact differently depending on culture conditions. Previously reported antagonistic effects of bedaquiline on imipenem were limited to conditions with actively multiplying bacteria and/or the presence of Tween 80, whereas the combination was additive or indifferent against nutrient-starved and intracellular M. abscessus, where promising bactericidal activity of the combination suggests it may have a role in future treatment regimens.

DOI: 10.1128/AAC.01545-21
PMID: 34516254

60. **Design and synthesis of 2-(2-isonicotinoylhydrazineylidene)propanamides as InhA inhibitors with high antitubercular activity.**

Eur J Med Chem. 2021 Nov 5;223:113668. doi: 10.1016/j.ejmech.2021.113668. Epub 2021 Jun 23.

Pflégr V(1), Horváth L(2), Stolaříková J(3), Pál A(4), Korduláková J(4), Bősze S(2), Vinšová J(1), Krátký M(5).

Based on successful antitubercular isoniazid scaffold we have designed its "mee-too" analogues by a combination of this drug linked with substituted anilines through pyruvic acid as a bridge. Lipophilicity important for passive diffusion through impenetrable mycobacterial cell wall was increased by halogen substitution on the aniline. We prepared twenty new 2-(2-isonicotinoylhydrazineylidene)propanamides that were assayed against susceptible Mycobacterium tuberculosis H37Rv, nontuberculous mycobacteria, and also multidrug-resistant tuberculous strains (MDR-TB). All the compounds showed excellent activity not only against Mtb. (minimum inhibitory concentrations, MIC, from ≤0.03 μM), but also against M. kansasii (MIC ≥2 μM). The most active molecules have CF3 and OCF3 substituent in the position 4 on the aniline ring.

MIC against MDR-TB were from 8 μM. The most effective derivatives were used for the mechanism of action investigation. The treatment of Mtb. H37Ra with tested compounds led to decreased production of mycolic acids and the strains overproducing InhA were more resistant to them. These results confirm that studied compounds inhibit the enoyl-acyl carrier protein reductase (InhA) in mycobacteria. The compounds did not show any cytotoxic and cytostatic activity for HepG2 cells. The amides can be considered as a promising scaffold for antitubercular drug discovery having better antimicrobial properties than original isoniazid together with a significantly improved pharmaco-toxicological profile.

DOI: 10.1016/j.ejmech.2021.113668
PMID: 34198149 [Indexed for MEDLINE]

61. **Decreased methylenetetrahydrofolate reductase activity leads to increased sensitivity to para-aminosalicylic acid in Mycobacterium tuberculosis.**

Antimicrob Agents Chemother. 2021 Nov 15:AAC0146521. doi: 10.1128/AAC.01465-21. Online ahead of print.

Yu JF(1)(2), Xu JT(1)(2), Yang SS(1), Gao MN(3), Si HR(1)(2), Xiong DY(1)(2), Gu J(1), Wu ZL(4), Zhou J(4), Deng JY(1)(5).

Tuberculosis (TB), caused by Mycobacterium tuberculosis (M. tuberculosis), is one of the most fatal diseases in the world. Methylenetetrahydrofolate reductase (MTHFR) catalyzes the production of 5-methyltetrahydrofolate (5-CH3-THF), which is required for the de novo biosynthesis of methionine in bacteria. In this study, we identified Rv2172c as an MTHFR in M. tuberculosis through in vitro and in vivo analyses and determined that the protein was essential for the in vitro growth of the bacterium. Subsequently, we constructed rv2172c R159N and L214A mutants in M. tuberculosis, and found that these mutants were more sensitive to the antifolates para-aminosalicylic acid (PAS) and sulfamethoxazole (SMX). Combining biochemical and genetic methods, we found that rv2172c R159N or L214A mutation impaired methionine production, leading to increased susceptibility of M. tuberculosis to PAS, which was largely restored by adding exogenous methionine. Moreover, overexpression of rv2172c in M. tuberculosis could increase methionine production and lead to PAS resistance. This research was the first to identify an MTHFR in M. tuberculosis and revealed that the activity of this enzyme was associated with susceptibility to antifolates. These findings have particular value for anti-tubercular drugs design for the treatment of drug-resistant TB.

DOI: 10.1128/AAC.01465-21
PMID: 34780266

62. **Correction: Cost comparison of nine-month treatment regimens with 20-month standardized care for the treatment of rifampicin-resistant/multi-drug resistant tuberculosis in Nigeria.**

PLoS One. 2021 Oct 28;16(10):e0259492. doi: 10.1371/journal.pone.0259492. eCollection 2021.

Bada FO, Blok N, Okpokoro E, Dutt S, Akolo C, Dakum P, Abimiku A.

Erratum for
PLoS One. 2020 Dec 1;15(12):e0241065.

[This corrects the article DOI: 10.1371/journal.pone.0241065.].

DOI: 10.1371/journal.pone.0259492
PMCID: PMC8553159
PMID: 34710200

63. **UNITE4TB: a new consortium for clinical drug and regimen development for TB.**

Int J Tuberc Lung Dis. 2021 Nov 1;25(11):886-889. doi: 10.5588/ijtld.21.0515.

Boeree MJ(1), Lange C(2), Thwaites G(3), Paton N(4), de Vrueh R(5), Barros D(6), Hoelscher M(7).

DOI: 10.5588/ijtld.21.0515
PMCID: PMC8544922
PMID: 34686229 [Indexed for MEDLINE]

64. **Comparative Performance of Genomic Methods for the Detection of Pyrazinamide Resistance and Heteroresistance in Mycobacterium tuberculosis.**

J Clin Microbiol. 2021 Nov 10:JCM0190721. doi: 10.1128/JCM.01907-21. Online ahead of print.

Whitfield MG(1)(2)(3), Engelthaler DM(4), Allender C(4), Folkerts M(4), Heupink TH(2), Limberis J(5), Warren RM(3), Van Rie A(2), Metcalfe JZ(5).

Background: Pyrazinamide is an important component of both drug-susceptible and drug-resistant tuberculosis treatment regimens. Although approximately 50% of rifampicin resistant isolates are also resistant to pyrazinamide, pyrazinamide susceptibility testing is not routinely performed due to the challenging nature of the assay. We investigated the diagnostic accuracy of genotypic and phenotypic methods, and explored the occurrence of pyrazinamide heteroresistance. Methods: We assessed pyrazinamide susceptibility among 358 individuals enrolled in the South African EXIT-RIF cohort using Sanger and targeted deep sequencing (TDS) of the pncA gene, whole genome sequencing (WGS), and phenotypic drug-susceptibility testing. We calculated the diagnostic accuracy of the different methods, and investigated the prevalence and clinical impact of pncA heteroresistance. True pyrazinamide susceptibility status was assigned to each isolate using the Koser classification and expert rules. Results: We observed 100% agreement across genotypic methods for detection of pncA fixed mutations, only TDS confidently identified three isolates (0.8%) with minor variants. For the 355 (99.2%) isolates that could be assigned true pyrazinamide status with confidence, phenotypic DST had a sensitivity of 96.5% (95% CI: 93.8-99.3%) and specificity of 100% (95% CI: 100-100%); both Sanger sequencing and WGS had a sensitivity of 97.1% (95% CI: 94.6-99.6%) and specificity of 97.8% (95% CI: 95.7-99.9%); and TDS, sensitivity of 98.8% (95% CI: 97.2-100%) and specificity of 97.8% (95% CI: 95.7-99.9%). Conclusions: We demonstrate high sensitivity and specificity for pyrazinamide susceptibility testing among all assessed genotypic methods. The prevalence of pyrazinamide heteroresistance in Mtb isolates was lower than that identified for other first-line drugs.

DOI: 10.1128/JCM.01907-21
PMID: 34757831

65. **Individualized Treatment Duration in Tuberculosis Treatment: Precision versus Simplicity.**

Am J Respir Crit Care Med. 2021 Nov 1;204(9):1013-1014. doi: 10.1164/rccm.202107-1744ED.

Adjobimey M(1)(2), Behr MA(3)(4)(5), Menzies D(3)(4)(5).

Comment on
Am J Respir Crit Care Med. 2021 Nov 1;204(9):1086-1096.

DOI: 10.1164/rccm.202107-1744ED

PMID: 34432615 [Indexed for MEDLINE]

66. **Discovery of Inhibitors for Mycobacterium Tuberculosis Peptide Deformylase Based on Virtual Screening in Silico.**

Mol Inform. 2021 Oct 27:e2100002. doi: 10.1002/minf.202100002. Online ahead of print.

Li X(1), Jiang Q(2), Yang X(1).

Tuberculosis has been the serious disease threatening human health and public safety due to the emergence of MDR and XDR-TB. Mycobacterium tuberculosis peptide deformylase (MtPDF) is a valuable target for antituberculotics. In order to discover new potential inhibitor candidates of MtPDF as leads for antituberculotics, Discovery Studio (DS) 2019 was used to perform molecular docking for virtual screening in silico with the bioactive compound library-I (L1700) against MtPDF. Six compounds with high docking scores and favourable ligand-protein interactions by LibDock and CDOCKER were selected for the evaluation of the inhibition potencies against MtPDF and Mycobacterium smegmatis. GST-6×His tagged MtPDF was recombinant expressed and purified firstly by Glutathione Sepharose 4B, and secondly by Ni Sepharose 6 FF after the cleavage of human rhinovirus 3C protease. These compounds showed IC50 values from 0.5 μmol/L to 112 μmol/L against MtPDF, among which CUDC-101 bearing hydroxamic acid exhibited IC50 of 0.5 μmol/L on MtPDF and MIC against Mycobacterium smegmatis of 32 μg/mL, and Ixazomib Citrate with IC50 of 63 μmol/L and MIC of 16 μg/mL. CUDC-101 and Ixazomib Citrate are promising as the potential leads for antituberculotics.

DOI: 10.1002/minf.202100002
PMID: 34708566

67. **Fluoroquinolone susceptibility in first-line drug-susceptible M. tuberculosis isolates in Lima, Peru.**

BMC Res Notes. 2021 Nov 14;14(1):413. doi: 10.1186/s13104-021-05832-0.

Schwalb A(1), Cachay R(2), Meza E(2), Cáceres T(2), Blackman A(3), Maruri F(3), Sterling TR(3), Gotuzzo E(2).

OBJECTIVE: To determine at two distinct time points the prevalence of resistance

to ofloxacin (OFX), the representative class drug of fluoroquinolones (FQs), in M. tuberculosis isolates susceptible to first-line drugs.
RESULTS: There were 279 M. tuberculosis isolates from the two cohorts (2004-2005: 238 isolates; 2017: 41 isolates) that underwent OFX drug-susceptibility testing (critical concentration: 2 μg/ml). Of 238 isolates in Cohort 1, no resistance to OFX was detected (95% CI 0-0.016); likewise, in Cohort 2, no resistance to OFX was detected in 41 isolates (95% CI 0-0.086). Our findings suggest that FQ use remains a viable option for the treatment of first-line drug-susceptible TB in Peru.

DOI: 10.1186/s13104-021-05832-0
PMCID: PMC8591909
PMID: 34776013 [Indexed for MEDLINE]

68. **Is a high dose sufficient to achieve high isoniazid exposure in children affected by multidrug-resistant TB?**

Int J Tuberc Lung Dis. 2021 Nov 1;25(11):879-880. doi: 10.5588/ijtld.21.0451.

Alffenaar JC(1), Marais BJ(2), Thomas TA(3).

DOI: 10.5588/ijtld.21.0451
PMID: 34686227 [Indexed for MEDLINE]

69. **Correlation of inhA mutations and ethionamide susceptibility: Experience from national reference center for tuberculosis.**

Lung India. 2021 Nov-Dec;38(6):520-523. doi: 10.4103/lungindia.lungindia_120_21.

Sarin R(1), Bhalla M(2), Kumar G(2), Singh A(2), Myneedu VP(2), Singhal R(2).

BACKGROUND: Detection of ethionamide (ETH) resistance is crucial as it is part of antitubercular regime. It is crucial to examine the role of inhA gene mutations as a surrogate marker for the detection of ETH resistance, in the Indian context. The present retrospective study was designed with this objective.
SUBJECTS AND METHODS: The study was conducted in National Reference Laboratory within the tertiary care institute from January 1, 2018, to June 30, 2019, over 18 months duration. A total of 6612 sputum samples from presumptive multidrug-resistant tuberculosis (TB) patients were received from four districts

of Delhi, outdoor and inpatients. Line probe assay (LPA) was performed for smear-positive or culture-positive samples for Mycobacterium tuberculosis. All isolates found to be INH resistant by LPA were cultured and phenotypic susceptibility to ETH was conducted for selected isolates as per the guidelines. RESULTS: A total of 246 isolates were analyzed, for which phenotypic susceptibility to ETH and mutations in inhA were available. ETH resistance was detected among 87/108 (80.5%) isolates with inhA mutation. Sensitivity and specificity of inhA mutation for detection of ETH resistance were 80.5% and 83.8%, respectively. No inhA mutation was detected in 29/116 (25%) ETH-resistant isolates in our study, whereas ETH was found to be phenotypically susceptible in spite of the presence of inhA mutation among 21/130 (16.1%) isolates. CONCLUSIONS: Mutations in inhA gene in LPA predict ETH resistance with fairly good sensitivity and specificity. However, it is imperative to perform phenotypic detection of ETH resistance at proper concentration, in addition to detecting inhA mutation.

DOI: 10.4103/lungindia.lungindia_120_21
PMID: 34747732

70. **Exploring disordered loops in DprE1 provides a functional site to combat drug-resistance in Mycobacterium strains.**

Eur J Med Chem. 2021 Oct 20;227:113932. doi: 10.1016/j.ejmech.2021.113932. Online ahead of print.

Liu J(1), Dai H(2), Wang B(3), Liu H(4), Tian Z(5), Zhang Y(6).

As an anti-tuberculosis target, DprE1 contains two flexible loops (Loop I and Loop II) which have never been exploited for developing DprE1 inhibitors. Here Leu317 in Loop II was discovered as a new functional site to combat drug-resistance in Mycobacterium strains. Based on TCA1, LZDT1 was designed to optimize the hydrophobic interaction with Leu317. A subsequent biochemical and cellular assay displayed increased potency of LZDT1 in inhibiting DprE1 and killing drug-sensitive/-resistant Mycobacterium strains. The improved activity of LZDT1 and its analogue LZDT2 against multidrug resistant tuberculosis was particularly highlighted. For LZDT1, its enhanced interaction with Leu317 also impaired the drug-insensitivity of DprE1 caused by Cys387 mutation. A new nonbenzothiazole lead (LZDT10) with reduced Cys387-dependence was further produced by optimizing interactions with Leu317, improvement directions for LZDT10 were discussed as well. Our research underscores the value of potential functional sites in disordered loops, and affords a feasible way to develop these functional sites into opportunities for drug-resistance management.

DOI: 10.1016/j.ejmech.2021.113932
PMID: 34700267

71. **Impact of the bacillary load on the accuracy of rifampicin resistance results by Xpert(®) MTB/RIF.**

Int J Tuberc Lung Dis. 2021 Nov 1;25(11):881-885. doi: 10.5588/ijtld.21.0564.

Ocheretina O(1), Brandao AP(2), Pang Y(3), Rodrigues C(4), Banu S(5), Ssengooba W(6), Dolinger DL(7), Salfinger M(8), Ngabonziza JCS(9), Köser CU(10).

DOI: 10.5588/ijtld.21.0564
PMID: 34686228 [Indexed for MEDLINE]