

Bedaquiline for treatment of non-tuberculous mycobacteria (NTM): a systematic review and meta-analysis

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Background: Non-tuberculous mycobacteria (NTM) infections are increasing in incidence and associated mortality. NTM are naturally resistant to a variety of antibiotics, complicating treatment. We conducted a literature assessment on the efficacy of bedaquiline in treating NTM species *in vitro* and *in vivo* (animal models and humans); meta-analyses were performed where possible.

Method: Four databases were searched using specific terms. Publications were included according to predefined criteria. Bedaquiline's impact on NTM *in vitro*, MICs and epidemiological cut-off (ECOFF) values were evaluated. A meta-analysis of bedaquiline efficacy against NTM infections in animal models was performed. Culture conversion, cure and/or relapse-free cure were used to evaluate the efficacy of bedaquiline in treating NTM infection in humans.

Results: Fifty studies met the inclusion criteria: 33 assessed bedaquiline's impact on NTM *in vitro*, 9 in animal models and 8 in humans. Three studies assessed bedaquiline's efficacy both *in vitro* and *in vivo*. Due to data paucity, an ECOFF value of 0.5 mg/mL was estimated for *Mycobacterium abscessus* only. Meta-analysis of animal studies showed a 1.86× reduction in bacterial load in bedaquiline-treated versus no treatment within 30 days. In humans, bedaquiline-including regimens were effective in treating NTM extrapulmonary infection but not pulmonary infection.

Conclusions: Bedaquiline demonstrated strong antibacterial activity against various NTM species and is a promising drug to treat NTM infections. However, data on the genomic mutations associated with bedaquiline resistance were scarce, preventing statistical analyses for most mutations and NTM species. Further studies are urgently needed to better inform treatment strategies.

Introduction

Non-tuberculous mycobacteria (NTM) is a heterogeneous group of environmental microorganisms that comprise more than 193 species (<https://www.bacterio.net/genus/mycobacterium>). NTM have the capacity to cause a variety of diseases in humans such as TB-like pulmonary or extrapulmonary disease, cervical lymphadenitis, visceral and disseminated disease in immunocompromised individuals.^{1,2} NTM infections are a health concern worldwide due to increasing incidence and associated mortality rates.³ *Mycobacterium avium* complex (MAC) and *Mycobacterium abscessus* are the most common pathogens associated with pulmonary NTM diseases, accounting for >90% of all reported

cases.⁴ Diagnosis of NTM infection is complicated due to the large number of NTM species, the overlap between the symptoms of TB and NTM disease, and the similar microscopic morphology of NTM to *Mycobacterium tuberculosis*. Treatment of NTM disease is difficult due to the limited number of therapeutic options^{1,5} as NTM species are naturally resistant to several available antibiotics.⁶

Following favourable treatment outcomes in several clinical and preclinical trials, the US FDA approved bedaquiline in 2012 for treatment of TB caused by infection with drug-resistant *M. tuberculosis*.⁷ In 2012, the WHO listed bedaquiline as one of the three core drugs for treatment of rifampicin-resistant TB.

A limited number of studies have investigated the susceptibility of NTM species to bedaquiline^{6,8} and have assessed the

efficacy of bedaquiline in treating NTM diseases in animal models and humans.⁹⁻¹¹ We aimed to systematically review the literature on the *in vitro* susceptibility of NTM to bedaquiline, including the distribution of MICs, and the efficacy of bedaquiline for treatment of NTM disease in animal and human studies. Moreover, we generated a profile of bedaquiline-resistant mutations identified in various NTM species.

Methods

This systematic review was conducted according to the PRISMA and meta-analysis guidelines¹² (PROSPERO CRD42020179792).

Search strategy and selection criteria

We searched four databases: PubMed, Web of Science, Scopus and PubMed European using the search terms: (bedaquiline OR diaryl quinolines OR diarylquinolines OR BDQ OR TMC207 OR R207910 OR Sirturo) AND (nontubercul* mycobacterium OR nontubercul* mycobacteria OR non-tubercul* mycobacterium OR non-tubercul* mycobacteria OR NTM OR atypical mycobacteria OR Mycobacteria other than tuberculosis OR MOTT) AND (abscessus OR avium complex OR chelonae OR fortuitum OR kansasii OR marinum OR scrofulaceum OR smegmatis OR ulcerans OR xenopi OR intracellulare), without language or date restrictions. The last search was conducted in December 2022.

After removing duplicate studies, article titles, abstracts and full texts were independently reviewed by three authors (S.O., M.K. and M.G.W.), using defined inclusion criteria as follows: (i) studies reporting on phenotypic and/or genotypic susceptibility to bedaquiline in NTM species using *in vitro* or *in vivo* method(s); and (ii) studies reporting on the efficacy of bedaquiline for treatment of NTM disease, in animal models or human studies. Reviews, conference articles and book chapters were excluded. Studies with both NTM and *M. tuberculosis* isolates but no stratification by mycobacterial species or microbiological results for individual isolates, as well as articles that discussed the use of bedaquiline for NTM but did not present any primary data, were also excluded. The reference lists of included articles were reviewed for any further relevant publications. When required, conflicts were settled by discussion between the three authors.

Data extraction

Data extraction was performed by one author (S.O.) and reviewed by another author (M.K.) to avoid errors. In case of disagreement between the two authors, a third author was consulted (M.G.W.).

For *in vitro* studies, the data extracted from each study included year of publication, first author, number of isolates tested (overall and by NTM species), study design, method of drug susceptibility testing (DST) used, bedaquiline MIC concentrations tested, bedaquiline MIC of NTM strains, reported classification as resistant or susceptible, presence of variants in genes that may confer resistance to bedaquiline, and sequencing method. Given the scarcity of data from NTM species, data from both clinical and non-clinical isolates were included.

For human and animal model studies, the data extracted from each study included the year of publication, first author, study design, study location, number of patients/animals included in the study, drug regimen, bedaquiline doses used, bedaquiline treatment duration, route of delivery, treatment outcome, follow-up duration, and participant demographics (age, sex and type of disease). When no raw data were provided, authors were contacted, otherwise data extraction (mean \pm standard deviation) from graphs was performed using WebPlotDigitizer (version 4.3).¹³ However, some data were difficult to extract using software, necessitating manual interpretation. Where data were provided as mean

\pm standard error of the mean (SEM), standard deviation (SD) was obtained by multiplying the square root of the sample size.

Quality assessment and risk of bias

Two authors (S.O. and M.G.W.) assessed the risk of bias and the quality of individual studies independently using the Quality Assessment of Diagnostic Accuracy Studies tool¹⁴ for *in vitro* studies, the SYstematic Review Centre for Laboratory animal Experimentation (SYRCL) tool for animal model studies (S.O. and M.G.W.)¹⁵ and the Joanna Briggs Institute 2017 Critical Appraisal Checklist for case series and case reports.¹⁶ Disagreement between the two authors was resolved by consulting a third author (M.K. for *in vitro* and human studies, and M.B.N. for animal studies).

Data analysis

Data analysis for *in vitro* studies and meta-analysis was performed by S.O. and confirmed by Dr Michael McCaul.

In vitro studies

Only isolates with clear MIC₉₉ values and one replicate per isolate were included in the analysis and distribution of WT isolates (excluding non-WT, reference and non-clinical strains) depicted graphically for various NTM species with 50 WT isolates being the minimum number used for a histogram. MIC is defined as the lowest concentration of an antimicrobial agent that inhibits the growth of 99% of an organism. MIC₉₉ was represented as MIC. The most frequent MIC value was used to define the mode. The epidemiological cut-off values (ECOFFs) and/or tentative ECOFF (T-ECOFF) values were estimated visually and/or statistically by using the ECOFFinder program¹⁷ and following EUCAST guidelines.¹⁸ Isolates reported with MIC values of 'lower or equal to' (\leq) were considered as equal to the particular MIC reported for simplicity and for the purpose of visualizing the histogram (e.g. ≤ 0.016 was reported as =0.016).

Due to the paucity of data related to the genomic mutations associated with phenotypic bedaquiline resistance, a descriptive analysis was performed only.

Animal model studies

To evaluate the treatment outcome 'decrease in bacterial load' expressed as log₁₀ cfu, meta-analysis was performed using Cochrane Collaboration Review Manager Software version 5.4.1.¹⁹ Due to the variations in mouse strains between animal model studies, the summary effect with 95% CI was calculated using the random-effects model. To account for different treatment durations and variations between studies in bacterial strains/subspecies, we converted the analysis to standardized mean differences (SMDs) and 95% CI was used for meta-analysis of treatment for up to 30 days.²⁰⁻²² Results were considered statistically significant at $P < 0.05$. Heterogeneity was measured using I^2 (with χ^2 and 95% CI) and assessed and interpreted according to the guidelines outlined in the Cochrane Handbook for Systematic Reviews of Interventions.²³ Where data were insufficient to perform meta-analysis, a descriptive analysis was considered instead.

Human studies

The efficacy of bedaquiline for treatment of NTM diseases was measured in terms of (time to) culture conversion, cure and/or relapse-free cure. Cure was defined as treatment completed with three or more consecutive negative cultures and symptom improvement with no relapse reported within the study period. Relapse is defined as reverting to culture positivity after initial successful treatment with culture conversion. Treatment failure was defined as the lack of three consecutive negative cultures, lack of symptom improvement, evidence of additional acquired resistance to the drugs, or adverse drug reactions (ADRs).

Results

Search results

We identified 374 records through the preliminary search. Following deduplication, 269 unique scientific research articles remained for potential inclusion in the review. Screening of the title, abstract and full text identified 50 eligible scientific articles reporting on the effect of bedaquiline on various NTM isolates

(Figure 1). Thirty-three studies reported on *in vitro* experiments^{6,8,24-54} (Table 1), while only nine animal model studies^{9,10,55-61} and eight human studies^{11,62-68} (Table 3) were found, with three studies assessing bedaquiline efficacy both *in vitro* and *in vivo*. Thus, these three studies^{61,62,68} were added to the *in vitro* analysis to compile a total of 36 studies conducting *in vitro* analysis. Of the 36 *in vitro* studies, 13 studies performed genotypic analysis.

Search Results

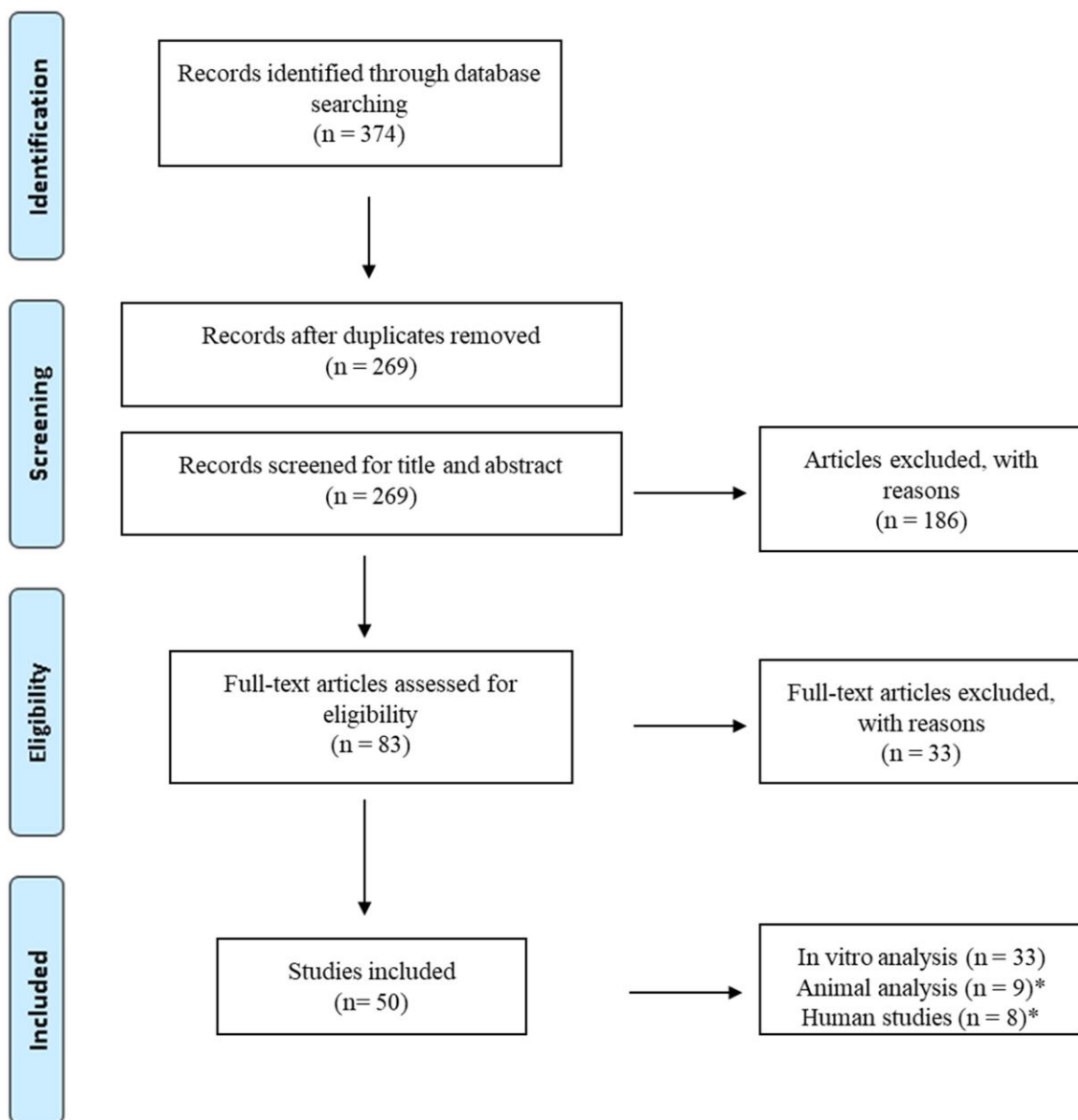


Figure 1. Flow diagram of studies selection. *One animal study and two human studies performed *in vitro* analysis and were included in our *in vitro* analysis.

Table 1. Characteristics of *in vitro* studies

Study	Total no. of isolates	Mycobacterium species	Growth	Isolate origin	Phenotypic assay	Genotypic assay	Gene investigated
Aguilar-Ayala et al. (2017) ⁶	18	<i>M. abscessus</i> , <i>M. chelonae</i> , <i>M. cosmeticum</i> , <i>M. duvalii</i> , <i>M. flavescens</i> , <i>M. fortuitum</i> , <i>M. franklinii</i> , <i>M. mageritense</i> , <i>M. mucogenicum</i> , <i>M. neoaurum</i> , <i>M. parafortuitum</i> , <i>M. peregrinum</i> , <i>M. phlei</i> , <i>M. smegmatis</i> , <i>M. wolinskyi</i> <i>M. intracellulare</i>	Rapid Slow	CCUG strains and UCL strain Clinical	MBC MIC CAMHB	Luria broth, REMA Sanger sequencing	<i>atpE</i>
Alexander et al. (2017) ⁶²	25	<i>M. intracellulare</i>	Slow	Clinical	MIC	WGS and targeted WGS	<i>atpE</i> and <i>mmT5 atpE</i>
Andries et al. (2005) ²⁴	26	<i>M. abscessus</i> , <i>M. avium</i> , <i>M. fortuitum</i> , <i>M. kansasii</i> , <i>M. marinum</i> , <i>M. smegmatis</i> , <i>M. ulcerans</i>	Rapid and slow	Non-clinical	MIC MBC	7H11, Mueller-Hinton plates	
Asami et al. (2021) ²⁵	70	<i>M. abscessus</i>	Rapid	Clinical	MIC	N/A	N/A
Brown-Elliott et al. (2017) ⁸	104	MAC and <i>M. avium</i>	Slow	Clinical and reference	MIC	N/A	N/A
Canitillon et al. (2022) ²⁶	2	<i>M. chimaera</i> , <i>M. abscessus</i>	Rapid and slow	Reference	MIC	REMA	N/A
Chew et al. (2021) ²⁷	211	<i>M. abscessus</i>	Rapid	Clinical	MIC	Sensititre plate	N/A
Chew et al. (2021) ²⁸	32	<i>M. fortuitum</i>	Rapid	Clinical	MIC	Sensititre plate	N/A
Dupont et al. (2017) ²⁹	38	<i>M. abscessus</i>	Rapid	Clinical and non-clinical	MIC	CAMHB	Sanger sequencing
Gumbo et al. (2020) ³⁰	20	<i>M. abscessus</i>	Rapid	Clinical and reference	MIC	CAMHB	Sequencing
Gutiérrez et al. (2019) ³¹	6	<i>M. abscessus</i>	Rapid	Non-clinical and reference	MIC	7H10	N/A
Huitric et al. (2007) ³²	50	<i>M. avium</i> , <i>M. intracellulare</i> , <i>M. chelonae</i> , <i>M. fortuitum</i> , <i>M. mageritense</i> , <i>M. phlei</i> , <i>M. vaccae</i> , <i>M. kansasii</i> , <i>M. malmoense</i> , <i>M. goodii</i> , <i>M. simiae</i> , <i>M. scrofulaceum</i> , <i>M. hiberniae</i> , <i>M. interjectum</i> , <i>M. szulgai</i> , <i>M. terrae</i> , <i>M. conspicuum</i> , <i>M. novocastrense</i> , <i>M. xenopi</i> , <i>M. shimoidei</i>	Rapid and slow	Clinical	MIC	7H10	Sanger sequencing
Kim et al. (2017) ³³	2	<i>M. abscessus</i>	Rapid	Non-clinical and reference	MIC	REMA	N/A
Kim et al. (2019) ⁵⁴	307	<i>M. abscessus</i> , <i>M. avium</i> , <i>M. intracellulare</i> , <i>M. kansasii</i>	Rapid and slow	Clinical	MIC	CAMHB	N/A
Li et al. (2018) ³⁴	197	<i>M. abscessus</i>	Rapid	Clinical	MIC	CAMHB	WGS
Lin et al. (2022) ³⁵	111	<i>M. avium</i> , <i>M. intracellulare</i> , <i>M. marsellense</i> , <i>M. colombiense</i> , <i>M. yongonense</i> , <i>M. vulneris</i> , <i>M. ardensis</i>	Slow	Clinical	MIC	Sensititre plate	<i>atpE</i> , MAB_4384 N/A

Litvinov et al. (2021) ³⁶	166	<i>M. avium</i> , <i>M. intracellulare</i>	Slow	Clinical	MIC	7H9	N/A	N/A
Lounis et al. (2008) ³⁷	1	<i>M. smegmatis</i>	Rapid	Reference	MIC	7H11, 7H11 + 5% BSA; LJ	N/A	N/A
Maeda et al. (2021) ³⁸	1	<i>M. smegmatis</i>	Rapid	Non-clinical	MIC	R liquid medium	WGS	<i>atpE</i> , <i>rpoB</i> , <i>prpE</i>
Martin et al. (2019) ³⁹	17	<i>M. avium</i> , <i>M. chimaera</i> , <i>M. intracellulare</i> , <i>M. kansasii</i> , <i>M. simiae</i> , <i>M. xenopi</i>	Slow	Clinical	MIC	REMA, 7H10	Sanger sequencing	<i>atpE</i>
Pang et al. (2016) ⁴⁰	685	<i>M. abscessus</i> , <i>M. avium</i> , <i>M. fortuitum</i> , <i>M. intracellulare</i> , <i>M. kansasii</i>	Rapid and slow	Clinical	MIC	CAMHB	Sanger sequencing	<i>atpE</i>
Ransom et al. (2022) ⁴¹	1	<i>M. grassiae</i>	Rapid	Clinical	MIC	Not reported	WGS	N/A
Richard et al. (2018) ⁴³	3	<i>M. abscessus</i>	Rapid	Non-clinical and reference	MIC	CAMHB	N/A	N/A
Richard et al. (2018) ⁴²	7	<i>M. abscessus</i>	Rapid	Non-clinical and reference	MIC	CAMHB	WGS	MAB_2299c
Richter et al. (2018) ⁴⁴	1	<i>M. abscessus</i>	Rapid	Non-clinical and reference	MIC	CAMHB	N/A	N/A
Ruth et al. (2019) ⁴⁵	28	<i>M. abscessus</i> , <i>M. avium</i> , <i>M. chimaera</i> , <i>M. fortuitum</i> , <i>M. intracellulare</i> , <i>M. kansasii</i> , <i>M. malmoense</i> , <i>M. simiae</i> , <i>M. xenopi</i>	Rapid and slow	Clinical and reference	MBC MIC	7H10, CAMHB	N/A	N/A
Sarathy et al. (2020) ⁶¹	12	<i>M. abscessus</i>	Rapid	Clinical and reference	MIC	7H9, CAMHB	N/A	N/A
Schulthess et al. (2022) ⁴⁶	62	<i>M. abscessus</i>	Rapid	Clinical and reference	MIC	CAMHB	N/A	N/A
Segala et al. (2012) ⁴⁷	37	<i>M. abscessus</i> , <i>M. fortuitum</i> , <i>M. smegmatis</i>	Rapid	Clinical, non-clinical and reference	MIC	7H11	Sequencing	<i>atpE</i>
Sorayah et al. (2019) ⁴⁸	17	<i>M. abscessus</i>	Rapid	Clinical	MIC	7H9	N/A	N/A
Srivastava et al. (2022) ⁴⁹	21	<i>M. kansasii</i>	Slow	Clinical and reference	MIC	7H9	N/A	N/A
Vesenbeckh et al. (2017) ⁵⁰	20	<i>M. avium</i> , <i>M. intracellulare</i>	Slow	Clinical	MIC	7H10	N/A	N/A
Vesenbeckh et al. (2017) ⁵¹	20	<i>M. abscessus</i>	Rapid	Clinical	MIC	7H10	N/A	N/A
Yu et al. (2019) ⁵²	260	<i>M. avium</i> , <i>M. intracellulare</i> , <i>M. kansasii</i> , <i>M. arupense</i> , <i>M. xenopi</i> , <i>M. szulgai</i> , <i>M. fuert</i> , <i>M. parascrofulaceum</i> , <i>M. terrae</i> , <i>M. malmoense</i> , <i>M. abscessus</i> , <i>M. fortuitum</i> , <i>M. goodii</i> , <i>M. chelonae</i> , <i>M. holsaticum</i> , <i>M. massiliense</i> , <i>M. asiaticum</i> , <i>M. celatum</i> , <i>M. chimaera</i> , <i>M. gastri</i> , <i>M. microti</i> , <i>M. nonchromogenicum</i> , <i>M. rhodesiae</i> , <i>M. scrofulaceum</i> , <i>M. triviale</i> , <i>M. agri</i> , <i>M. aichiense</i> , <i>M. aurum</i> , <i>M. austroafricanum</i> ,	Rapid and slow	Clinical and reference	MIC	CAMHB	N/A	N/A

Continued

Table 1. Continued

Study	Total no. of isolates	Mycobacterium species	Growth	Isolate origin	Phenotypic assay	Genotypic assay	Gene investigated
		<i>M. chitae</i> , <i>M. chubuense</i> , <i>M. cosmeticum</i> , <i>M. diernhoferi</i> , <i>M. fallax</i> , <i>M. farcinogenes</i> , <i>M. flavescens</i> , <i>M. gadium</i> , <i>M. gilvum</i> , <i>M. goodii</i> , <i>M. mucogenicum</i> , <i>M. neoaurum</i> , <i>M. obuense</i> , <i>M. parafortuitum</i> , <i>M. peregrinum</i> , <i>M. phlei</i> , <i>M. porcinum</i> , <i>M. pulveris</i> , <i>M. senegalense</i> , <i>M. septicum</i> , <i>M. simiae</i> , <i>M. sphagni</i> , <i>M. smegmatis</i> , <i>M. thermoresistibile</i> , <i>M. tokaiense</i> , <i>M. vaccae</i>	Rapid and slow	Clinical	CAMHB	N/A	N/A
Zhu et al. (2022) ⁵³	182	<i>M. kansasii</i> , <i>M. avium</i> , <i>M. intracellulare</i> , <i>M. abscessus</i> , <i>M. abscessus</i> subsp. <i>massiliense</i> , <i>M. agri</i> , <i>M. aichiense</i> , <i>M. aurum</i> , <i>M. austroafricanum</i> , <i>M. brumae</i> , <i>M. confluentis</i> , <i>M. chelonae</i> , <i>M. chitae</i> , <i>M. chubuense</i> , <i>M. cosmeticum</i> , <i>M. diernhoferi</i> , <i>M. fortuitum</i> , <i>M. fortuitum</i> subsp. <i>fortuitum</i> , <i>M. mucogenicum</i> , <i>M. neworleansense</i> , <i>M. obuense</i> , <i>M. parafortuitum</i> , <i>M. peregrinum</i> , <i>M. pulveris</i> , <i>M. senegalense</i> , <i>M. septicum</i> , <i>M. smegmatis</i> , <i>M. thermoresistibile</i> , <i>M. tokaiense</i> , <i>M. asiaticum</i> , <i>M. arosiense</i> , <i>M. celatum</i> , <i>M. chimaera</i> , <i>M. gastri</i> , <i>M. gadium</i> , <i>M. gordonae</i> , <i>M. interjectum</i> , <i>M. kubicae</i> , <i>M. marinum</i> , <i>M. microti</i> , <i>M. nonchromogenicum</i> , <i>M. parascrofulaceum</i> , <i>M. rhodesiae</i> , <i>M. scrofulaceum</i> , <i>M. szulgai</i> , <i>M. sphagni</i> , <i>M. shimoidei</i> , <i>M. terrae</i> , <i>M. triviale</i> , <i>M. xenopi</i> , <i>M. tuberculosis</i> (H37Rv)	Slow	Clinical	CAMHB	WGS	<i>atpE</i> ; <i>MAV_2512</i>
Zweijpfenning et al. (2019) ⁶⁸	3	<i>M. avium</i>	Slow	Clinical	CAMHB	WGS	<i>atpE</i> ; <i>MAV_2512</i>

LJ, Löwenstein-Jensen; N/A, not applicable.

Table 2. Characteristics of animal model studies

Study (year)	Species	BDQ MIC (mg/L)	Route of infection	Animal strain	Number of mice (N)	BDQ dosage (mg/kg)	Route of drug administration	Treatment regimen (n)	Duration of treatment and follow-up	Outcome measured by
Chauffour <i>et al.</i> (2016) ⁵⁵	<i>M. ulcerans</i>	N/R	Infecting two footpads	Female BALB/c/mice	60	25	Oral gavage	RFP+BDQ (60)	Up to 4 weeks and follow up till 28 weeks	Lesion index and cfu/footpad
Converse <i>et al.</i> (2019) ⁹	<i>M. ulcerans</i>	0.125	Infecting both hind footpads	BALB/c mice	116	25	Oral gavage	RIF+RFP+CLF+BDQ (29) RFP+BDQ+Q203 (29) RFP+CLF+BDQ+Q203 (29) CLF+BDQ+Q203 (29)	Up to 2 weeks and follow-up till 28 weeks	Lesion index and cfu/footpad
Ji <i>et al.</i> (2006) ⁵⁶	<i>M. ulcerans</i>	N/R	Infecting the left hind footpad subcutaneously	Female BALB/c/mice	40	25	Oral gavage	BDQ (20) RIF+BDQ (20)	Up to 8 weeks	cfu/footpad
Komm <i>et al.</i> (2021) ⁵⁷	<i>M. ulcerans</i>	N/R	Subcutaneously in both hind footpads	BALB/c mice and Fox Chase SCID Beige mice	59	25	Oral gavage	BDQ+Q203 (59)	Up to 10 days and follow up till 17 weeks	Lesion index and cfu/footpad
Le Moigne <i>et al.</i> (2020) ⁵⁸	<i>M. abscessus</i>	0.125	Intratracheally	C3HeB/FeJ mice	20	30	Oral gavage	BDQ (10) BDQ+IPM(10)	Up to 3 weeks	cfu/organ (lung, spleen and liver)
Lerat <i>et al.</i> (2014) ¹⁰	<i>M. abscessus</i>	0.5	Intravenously via tail vein	Nude mice	15	25	Oesophageal gavage	BDQ (15)	Up to 8 weeks	cfu/organ (lung, spleen and kidney)
Lounis <i>et al.</i> (2009) ⁵⁹	<i>M. avium</i>	N/R	Infected intraperitoneally	Female C57BL/6J mice	80	25	N/R	BDQ (20) BDQ+CLR (20) BDQ+AMK (20) BDQ+CLR+AMK (20)	Up to 16 weeks	cfu on the spleen
Obregón-Henao <i>et al.</i> (2015) ⁶⁰	<i>M. abscessus</i>	1 ± 0.01	Intravenously via tail	GKO-/- mice lack IFN and SCID mice	30	30	Subcutaneous injection	BDQ (15) BDQ+CLF (15)	Up to 2 weeks	cfu/organ (lung, spleen and liver)
Sarathy <i>et al.</i> (2020) ⁶¹	<i>M. abscessus</i>	N/R	Intranasal	Female NOD.CB17-Prkdcscid/NCRcrl mice	12	20	Oral gavage	BDQ (12)	Up to 2 weeks	cfu/organ (lung and spleen)

N, total number of treated mice in BDQ/BDQ-including regimen in the study; n, number of mice in each treatment group; N/R, not reported. RFP, rifampentine; BDQ, bedaquiline; RIF, rifampicin; Q203, telacebec; IPM, imipenem; CLR, clarithromycin.

Table 3. Characteristics of human studies

Study (year)	Study approach	Total no. of patients	No. of patients treated	Age (years)	Disease	HIV status	Mycobacterium species	Treatment status	In vitro BDQ MIC (mg/L)	Treatment regimen (n)	BDQ dose	Duration of treatment
Alexander et al. (2017) ⁶²	Case series	7	1	N/ 54	NB+C		<i>M. intracellulare</i>	Pre	0.004	BDQ+EMB+	200 mg (thrice weekly)	>6 months
			R	60	N			On	0.008	RFB+STR (1)		
			N/ 1	68	FN+C			Pre	0.004	BDQ+AMK+		
			R	71	NB+C			On	0.008	EMB+RFB (1)		
			N/ 1	64	FN+C			Pre	0.004	BDQ+AMK+		
			R	58	N			On	0.015	ATM+EMB+STR (1)		
			N/ 1	66	NB+C			Pre	0.004	BDQ+EMB+		
R	64	FN+C			On	2.00	RFB+STR (1)					
Chan et al. (2021) ⁶³	Case study	1	F	4	Mycobacterial calcaneal osteomyelitis	No	<i>M. abscessus</i>	Pre	0.015	BDQ+CLF (1)	100 mg (thrice weekly)	8 months
			F	20	Severe soft-tissue infection	No	<i>M. fortuitum</i> complex	Pre	<0.063	BDQ+LVX (1)	4.00 mg (orally once daily for 14 days)	3.5 month
Eiber et al. (2020) ⁶⁴	Case study	2	M	54	Disseminated NTM infection, presumed secondary to faecal abdominal cavity contamination	Yes	<i>M. abscessus</i>	Pre		BDQ+CLF+ATM+LZD (1)	400 mg/day (for 2 weeks, followed by 200 mg thrice monthly)	21 months
			M	30	Pyrexia, pancytopenia and lymphadenopathy	Yes	<i>M. avium</i>	Pre		BDQ+TDZ+CLF (1)	400 mg/day (for 2 weeks, followed by 200 mg thrice weekly)	14 months
Meybeck et al. (2021) ⁶⁶	Case study	1	F	54	Cutaneous and subcutaneous nodules, mediastinal lymph nodes with left pulmonary infiltrate.	Yes	<i>M. marinum</i>	Pre		BDQ+MFX (1)	400 mg/day (loading phase for 2 weeks daily followed by a continuation phase of 200 mg thrice weekly)	12 months
			M	41	Osteomyelitis and bacteraemia	No	<i>M. abscessus</i>	Pre	0.12	BDQ+OMC (1)	300 mg/day	24 months BDQ+OMC

Philly et al. (2015) ¹¹	Case series	10	1	M	36	N	<i>M. abscessus</i>	AMK+LZD+TGC+BDQ (1)	400 mg (four tablets of 100 mg once daily with food for the first 2 weeks followed by 200 mg as two tablets of 100 mg)	≥6 months
		1	M	31	N	<i>M. abscessus</i>	BDQ+ATM+AMK+TGC+MFX (1)		≥6 months	
		1	F	64	C	<i>M. abscessus</i>	CLR+MFX+ATM+DOX+AMK+TGC+BDQ (1)		≥6 months	
		1	F	65	N	<i>M. abscessus</i>	LZD+LVX+IPM+AMK+BDQ (1)		≥6 months	
		1	F ^a	58	N	<i>M. avium</i> complex (<i>M. intracellulare</i>)	RFB+EMB+STR+BDQ (1)		≥6 months	
		1	M ^a	54	C	<i>M. avium</i> complex (<i>M. intracellulare</i>)	STR+EMB+RFB+BDQ (1)		≥6 months	
		1	M ^a	64	FN+C	<i>M. avium</i> complex (<i>M. intracellulare</i>)	EMB+CLR+RFB+STR+BDQ (1)		≥6 months	
		1	F ^a	60	N	<i>M. avium</i> complex (<i>M. intracellulare</i>)	EMB+ATM+AMK+RFB+STR+BDQ (1)		≥6 months	
		1	M	65	C	<i>M. avium</i> complex	EMB+RFB+STR+BDQ (1)		≥6 months	
		1	F	73	C	<i>M. avium</i> complex	EMB+RIF+STR+BDQ (1)		≥6 months	
Zweijpfenning et al. (2019) ⁶⁸	Case study	1	F	50	NB	<i>M. avium</i>	BDQ+CLF+EMB+CLR (1)	400 mg/day for 2 weeks then 200 mg thrice weekly	12 months	
							Pre		<0.003	
							On		0.06	
							On		0.06	

M, male; F, female; NB, nodular bronchiectasis; C, cavitary; N, nodular; FN, fibronodular; RIF, rifampicin; AMK, amikacin; BDQ, bedaquiline; CLF, clofazimine; CLR, clarithromycin; IPM, imipenem; RFB, rifabutin; RIF, rifampicin; Q203, telacebec; MFX, moxifloxacin; OMC, omadacycline; TDZ, tedizolid; EMB, ethambutol; STR, streptomycin; RFB, rifabutin; AZM, azithromycin; LZD, linezolid; LVX, levofloxacin; TGC, tigecycline; DOX, doxycycline; pre: pre-treatment; on, on treatment.

^aCases reported in two studies.

Description of the included studies

In all 50 included studies, phenotypic MIC data for bedaquiline of 2777 (excluding duplicates) isolates were reported, including 2571 (92.5%) clinical isolates, 59 (2.1%) non-clinical isolates that were generated *in vitro*, 124 (4.5%) reference strains and 23 (0.8%) for which data on the isolate origin were not available. Out of these 2777 isolates, 1527 (55%) isolates were rapidly growing NTMs (43 species), while 1250 (45%) isolates were slow-growing NTMs (33 species).

M. abscessus species constituted most (84%) of the rapidly growing isolates (1288/1527 isolates, 29 studies), followed by *Mycobacterium fortuitum* (109 isolates, 7%, 9 studies). Among the slow-growing species, *Mycobacterium intracellulare* (452/1250 isolates, 36%, 11 studies), *M. avium* (336 isolates, 27%, 14 studies), *Mycobacterium kansasii* (246 isolates, 20%, 9 studies) and MAC (110 isolates, 9%, 2 studies) were the most frequently reported species. The remaining species were represented by ≤ 50 isolates each.

In vitro studies

The characteristics of studies included in *in vitro* analysis are presented in Table 1. In summary, bedaquiline DST was performed using MIC for 2763 isolates, of which 37 isolates underwent DST using minimum bactericidal concentration (MBC) as well. Bedaquiline MIC was assessed using several methods: CAMHB was the most common method used (17 studies) in which a total of 1995 (72.2%) isolates of different species were characterized, followed by Sensititre plates (345 isolates, 13%, 3 studies), 7H9 (213 isolates, 7.7%, 5 studies), 7H10 (96 isolates, 3.5%, 4 studies) and 7H11 (60 isolates, 2%, 2 studies). Resazurin microtitre assay (REMA), R medium, 7H11+5% BSA and Löwenstein-Jensen medium were used in various studies for the remaining isolates, with < 50 isolates tested by each method. MBC was assessed using three methods including 7H10, Luria broth agar, and Mueller-Hinton plates, but no method was applied to > 50 isolates. One study did not specify the media they used on one isolate (Table S1, available as [Supplementary data](#) at JAC Online).

A total of 13 (36%) studies performed genotypic analysis for 1008 isolates (951 clinical and 57 non-clinical) using different sequencing approaches. Sanger sequencing was the most common approach, used for 729 isolates (five studies), followed by WGS (237 isolates, six studies) including targeted sequencing of 25 isolates (one study). In addition, two studies reported on the sequencing of 42 isolates without describing the sequencing approach (Table 1).

Animal model studies

The characteristics of the nine animal studies included in the analysis are shown in Table 2. Six (67%) studies reported on using either bedaquiline alone, followed by combination with other antibiotics, with substantial variation across studies.^{10,56,58-61} Three studies (33%) reported on the effectiveness of bedaquiline in combination with different antibiotics.^{9,55,57}

Four (44%) and five (56%) studies, respectively, reported on the effect of bedaquiline alone or in a combined regimen in treating skin infection caused by *Mycobacterium ulcerans* species,^{9,55-57}

or disseminated infection caused by either *M. abscessus* species^{10,58,60,61} or *M. avium* species⁵⁹ (Table 2).

All the animal studies were performed in mice. However, mouse models, the total number of mice, age and sex of mice, the disease induction protocol, the route of drug administration and the treatment duration all varied across the studies (Table 2).

Human studies

Eight descriptive studies were identified that reported on the efficacy of bedaquiline in treating NTM-infected patients. All of the included studies used bedaquiline in combination with other antibiotics (different combinations of amikacin, ethambutol, rifabutin, clarithromycin, streptomycin, linezolid, tigecycline, azithromycin, moxifloxacin or omadacycline).

Of the eight human studies identified, four were case series^{11,62,65,67} and four were case reports (Table 3).^{63,64,66,68} Two of the studies were conducted by the same group^{11,62} and had an overlap of four patients infected with *M. intracellulare*. However, the analyses done by these studies were completely different. Although Alexander et al.⁶² reported that 16 cases were enrolled in the case series, the clinical data of only seven patients who experienced relapse were reported, while no clinical data of the remaining nine bedaquiline-treated patients were provided. Therefore, this review reports on results of bedaquiline treatment available for 20 patients (Figure S1).

Of the 20 treated patients, 14 patients had pulmonary disease, and the remaining 6 patients had extrapulmonary diseases, in which 5 had disseminated infection and 1 patient had a severe soft-tissue infection in the lower leg.⁶³ Studies varied in the NTM species investigated, the type of data provided, including bedaquiline DST data, and the measure of patient improvement. Details of the characteristics of the human studies are provided in Table 3.

Study quality and risk of bias

In vitro studies

Among studies included in the *in vitro* analysis, around 64% (23/36) showed a high risk of bias in the index test domain and index test applicability as these studies did not perform the index test. All studies showed unclear risk in the reference test due to either unclear blinding or unclear methodology (e.g. range of the MIC dilutions) used. Most of the studies showed low risk in the reference test applicability with only three studies that showed unclear risk due to unclear reporting of the method used (Figure 2 and Figure S2).

Animal model studies

An assessment of selection bias showed that in all studies (100%) it was unclear whether the animals were assigned to groups in a blinded manner (blinding allocation). In six studies (67%), it was unclear whether random allocation was employed. Five (56%) of the studies reported on group similarities at baseline. An assessment of the risk of performance bias demonstrated in all studies (100%) that it was unclear whether the animals were randomly housed, and whether the investigators were blinded during experiments. When evaluated for detection bias, all studies (100%) were unclear on random outcome

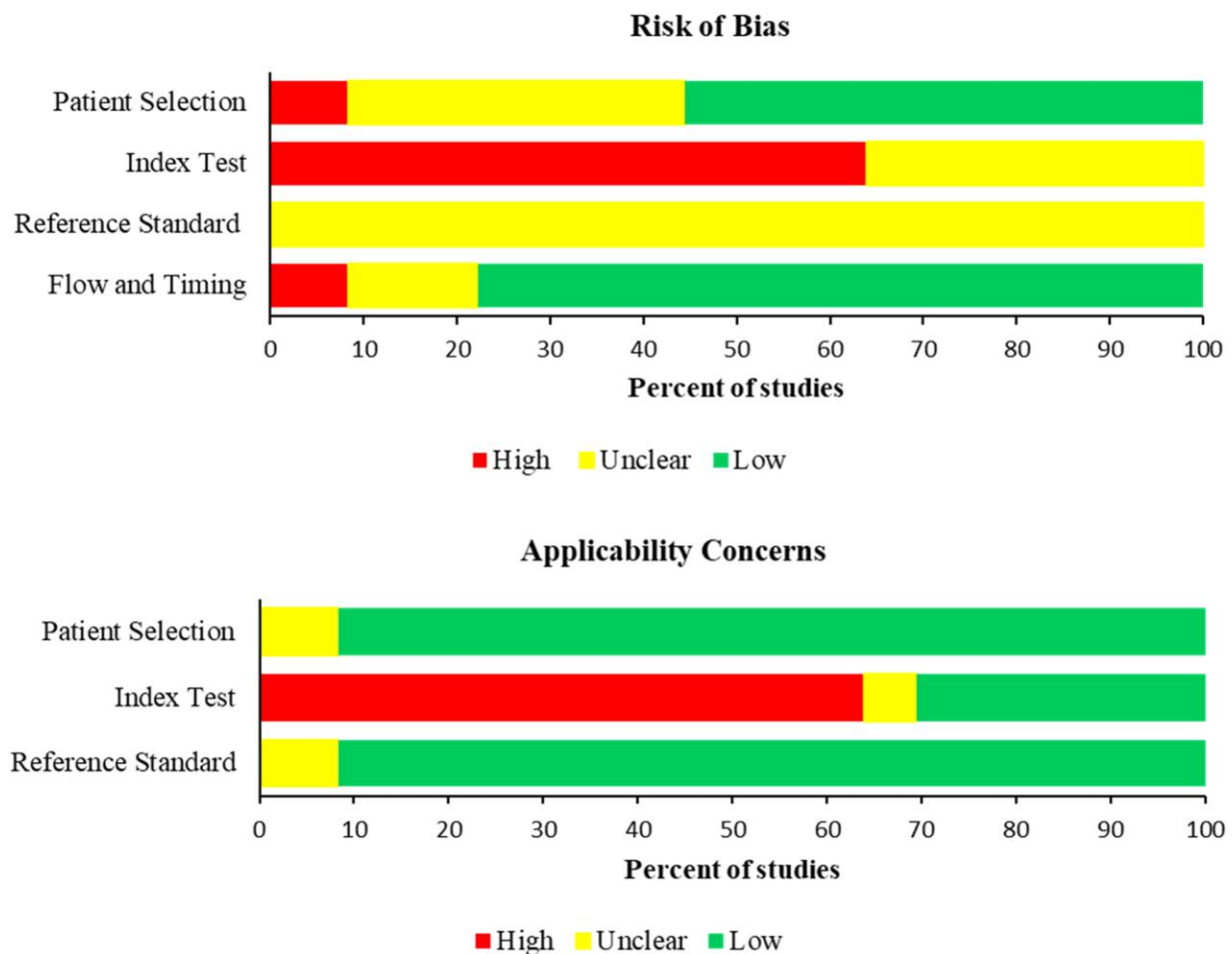


Figure 2. Risk of bias of the *in vitro* studies. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

assessment and whether the outcome assessor was blinded to group allocation. Six studies (67%) had an unclear attrition bias due to poor reporting of the number of experimental animals in methods and results sections, two studies (22%) had a high risk of attrition bias, and one study (11%) had a low risk of bias. The majority of studies (89%; 8/9) had a low risk of selective reporting bias (Figure 3 and Table S2).

Human studies

None of the case series studies reported the presenting site(s)/ clinic(s) clearly. Three (75%) of the case series were unclear about using consecutive inclusion of participants. Three case-series studies (75%) did not define the confounding factors and strategies to deal with it clearly. Two (50%) of the case-series studies were unclear about the outcome of assessment and follow-up of all the cases and one study was unclear about adverse events (Figure 4 and Table S3). The two case-report studies had low risk of bias in all domains. Both studies had clearly defined the inclusion criteria and identified confounding factors and treatment strategies were discussed. Both studies clearly described patients' history, clinical conditions, and diagnostic assessment methods. One case report did not describe patient demographics

clearly nor provide a clear timeline, and all of the reports were unclear about the confounding factors (Figure 5 and Table S4).

Data analysis

We analysed data from *in vitro* studies to assess the effect of bedaquiline by using MIC distribution histograms with the aim to propose ECOFF values for various NTM species. However, sufficient data were available for only two rapidly growing species (*M. abscessus* and *M. fortuitum*) and three slow-growing species (*M. avium*, *M. intracellulare*, *M. kansasii*). Data from other species were scarce and thus we were unable to analyse them.

The effect of bedaquiline in treating NTM species in *in vitro* studies

As MIC distribution can vary across platforms and between species, where data were available, we evaluated bedaquiline MIC distribution of various NTM species accordingly.

Bedaquiline MIC distributions in CAMHB CAMHB was the most commonly employed medium (19 studies) to define the phenotypic behaviour of 1996 (72%) isolates in the presence of bedaquiline (Table 1). The overall bedaquiline MIC range in CAMHB

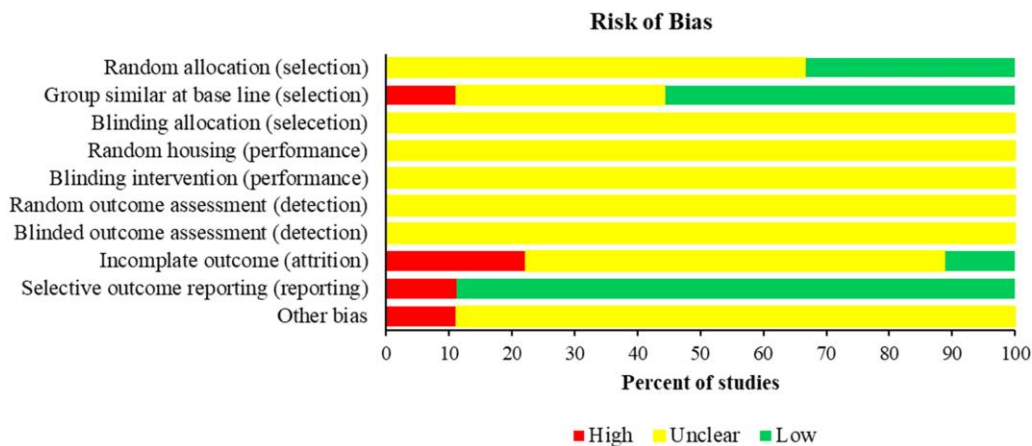


Figure 3. Quality assessment of animal model studies using SYRCL tool. This figure appears in colour in the online version of JAC and in black and white in the print version of JAC.

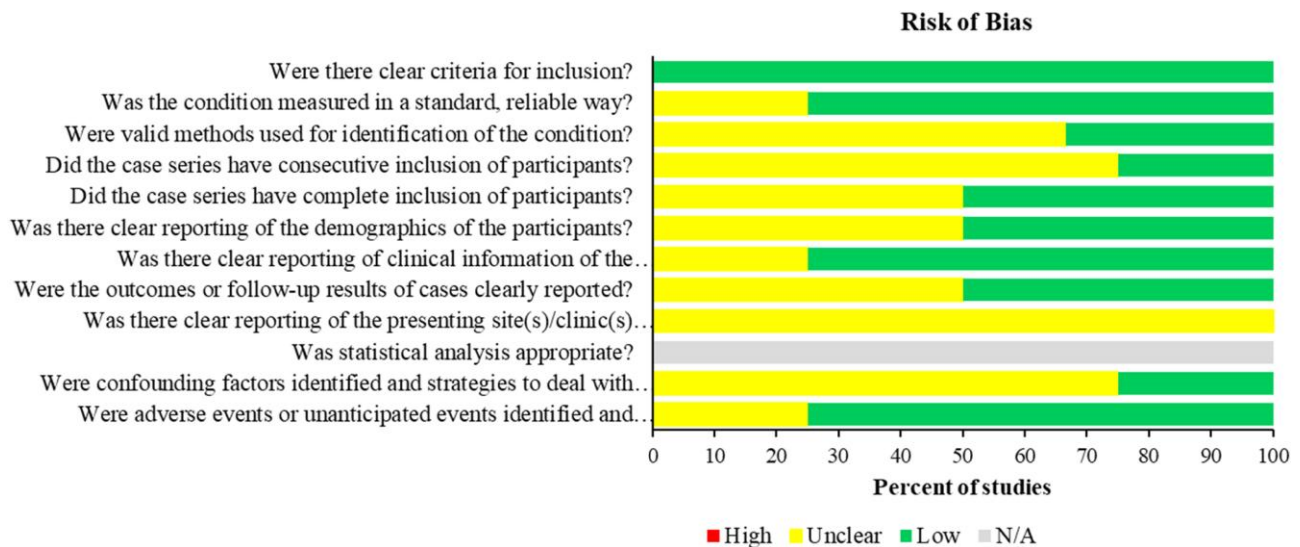


Figure 4. Risk of bias of case series. This figure appears in colour in the online version of JAC and in black and white in the print version of JAC.

across NTM species was between 0.008 and >32 mg/L. However, not all MIC histograms formed bimodal distributions, as shown below.

Rapidly growing mycobacteria (RGM)

Of 1996 isolates, 911 WT *M. abscessus* isolates from eight studies reporting MIC₉₉ values^{25,29,30,34,40,52-54} were included in the MIC distribution histogram (Figure 6a). The bedaquiline MIC distribution of these isolates ranged from 0.008 to >32 mg/L, with most of the isolates aggregated between 0.031 and 0.25 mg/L, and the mode at 0.062 mg/L with 29% (265/911) isolates. Around 7% (62/911) had an MIC > 16 mg/L. Visual inspection of the *M. abscessus* MIC distribution suggests that the cut-off value should be 1.0 mg/L (Figure 6a, blue arrow). Thus, 89% (812/911) of isolates were susceptible to bedaquiline, while 11% (99/911) were resistant.

MIC data of 56 (56/1681; 3%) *M. fortuitum* isolates from three studies^{40,45,52} were available and included in the MIC distribution histogram (Figure 6b). The MIC distribution ranged between 0.016 and >16.0 mg/L, with the majority (32%) of isolates aggregating at an MIC of 0.5 mg/L. Based on a visual evaluation of the histogram of *M. fortuitum* isolates, a cut-off value of 2.0 mg/L could be established, with 82% (46/56) susceptible and 18% (10/56) resistant to bedaquiline.

Slow-growing mycobacteria (SGM)

Using CAMHB, the majority of the isolates of the most common slow-growing NTM species aggregated between an MIC of 0.008 and 0.062 mg/L. Of the 155 isolates of *M. avium* from five studies,^{40,52-54,68} 60% (93/155) had an aggregated MIC (mode) of 0.016 mg/L, with the MIC of one isolate at <0.003 mg/L and another three isolates at ≥16 mg/L. Although the MIC distribution

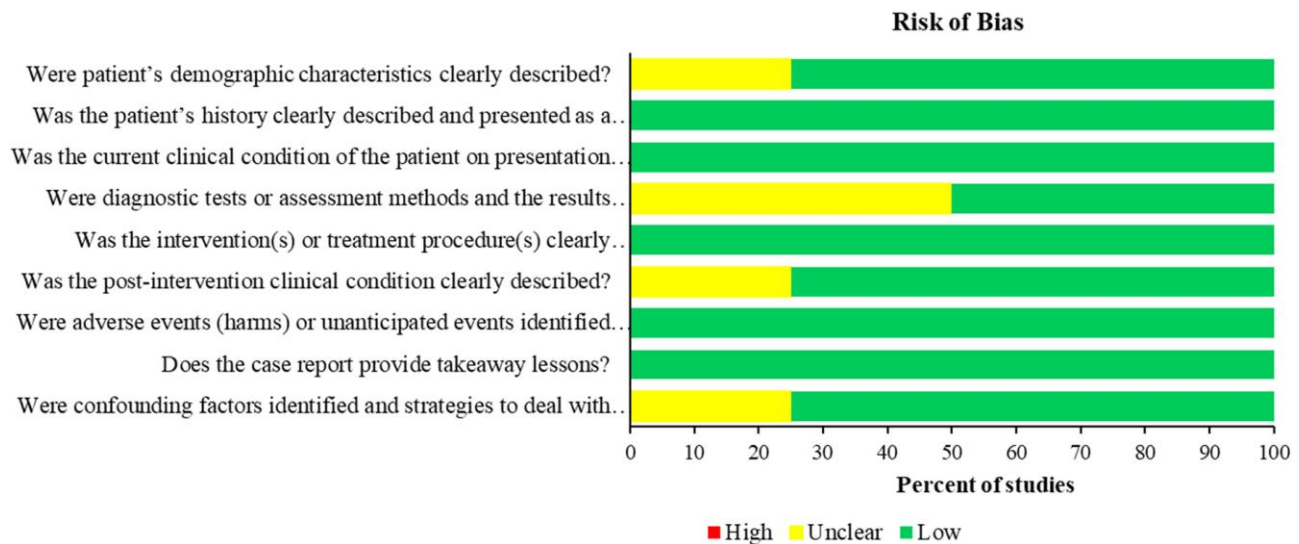


Figure 5. Risk of bias of case reports. This figure appears in colour in the online version of JAC and in black and white in the print version of JAC.

for *M. avium* was a skew to the right and did not form a clear bimodal distribution, a value of 0.125 mg/L can be proposed as a cut-off point. Applying this value, 86% (134/155) isolates were susceptible and 14% (21/155) were resistant (Figure 6c).

Two hundred and eighty-nine isolates of WT *M. intracellulare* were evaluated in six studies^{40,45,53,54,62} using CAMHB. In contrast to *M. avium*, the MIC histogram of *M. intracellulare* showed a bimodal distribution. However, the majority of *M. intracellulare* isolates (48%; 139/289) shared the same mode as *M. avium*, which was 0.016 mg/L. Visual inspection of the MIC histogram of *M. intracellulare* suggests a cut-off value of 0.25 mg/L. Applying this value, 81% (235/289) of *M. intracellulare* were susceptible and 19% (54/289) were resistant to bedaquiline (Figure 6d). Due to the small number of *M. intracellulare* and *M. avium* isolates, the MIC distribution was not compiled as one complex.

Data for *M. kansasii* revealed that 33% (73/220) of isolates from five studies had a modal MIC of 0.016 mg/L.^{40,45,52–54} A cut-off value of 0.25 mg/L could also be proposed for *M. kansasii* (Figure 6e).

Bedaquiline MIC distribution in Sensititre Two recent studies reported on the bedaquiline MIC distribution using Sensititre plates. One study reported on bedaquiline MIC distribution of 211 *M. abscessus* isolates, of which 146 were respiratory isolates,²⁷ using a Sensititre RAPMYCO plate and a customized broth microdilution testing panel (SGPNUHS1 plate) (Sensititre, Thermo Fisher Scientific, Waltham, MA, USA). Bedaquiline MIC ranged from 0.008 to 0.25 mg/L. The majority (96/211; 45%) of isolates had an MIC of 0.06 mg/L. The proposed T-ECOFF value of 99.0% was 0.5 mg/L. Using the same method, the same author reported that the bedaquiline MIC distribution of 32 isolates of *M. fortuitum* ranged between 0.004 and 0.015 mg/L, with the MIC mode at 0.008 mg/L (Figure S3).²⁸

The other study reported bedaquiline MIC distribution of a total of 111 isolates of five different species of MAC using the Sensititre Myco susceptibility plate for SGM (Thermo Fisher

Scientific). Bedaquiline MIC of *M. avium* and *M. intracellulare* ranges were 0.03–0.12 and 0.015–0.12 mg/L, respectively. The study showed that majority of MAC isolates were susceptible to bedaquiline and isolates from both species (*M. avium* and *M. intracellulare*) aggregated at an MIC of 0.06 mg/L.

Bedaquiline MIC distribution in 7H9 Two studies reported on MIC distribution of SGM species in 7H9.^{36,49} In one study, the bedaquiline MIC distributions of *M. avium* and *M. intracellulare* in 7H9 were mostly in the ranges 0.003–1.0 and 0.003–0.5 mg/L, respectively, with the MIC modes being 0.015 and 0.007 mg/L, respectively (Figure S3). The majority of *M. avium* (98/124; 79.0%) and *M. intracellulare* (37/42; 88.1%) isolates had a bedaquiline MIC of <0.03 mg/L. The T-ECOFF values for *M. avium* and *M. intracellulare* that were proposed by the authors were 0.12 and 0.06 mg/L, respectively. Using the proposed T-ECOFF value, only two isolates of *M. avium* and three isolates of *M. intracellulare* were found to be resistant to bedaquiline in 7H9.³⁶

The other study reported on bedaquiline MIC distribution of only 20 isolates of *M. kansasii*, in which most isolates ($n=20$) had bedaquiline MIC of 0.03 mg/L and only 2 isolates were resistant to bedaquiline with MIC of 2 mg/L (Figure S3).⁴⁹

ECOFF value determined by fitting a log-normal distribution (ECOFFinder)

MIC distributions from five studies only^{25,29,34,53,54} fitted EUCAST criteria and data of 471 isolates were included in ECOFFinder analysis. The estimated values of the ECOFF 95.0%, and 99.0% for *M. abscessus* were 0.25 and 0.5 mg/L, respectively (red arrow in Figure 6 and Figure S4). These values differ by one 2-fold dilution from the visual estimated values that are accepted. An MIC above the ECOFF 99.9% was categorized as resistance.^{69,70} Applying an ECOFF of 0.5 mg/L showed that 2% (8/471) of isolates were resistant.

None of the SGM MIC distributions met the EUCAST requirements for acceptable distributions since they were either truncated at the lower end or had too few WT observations (<15)

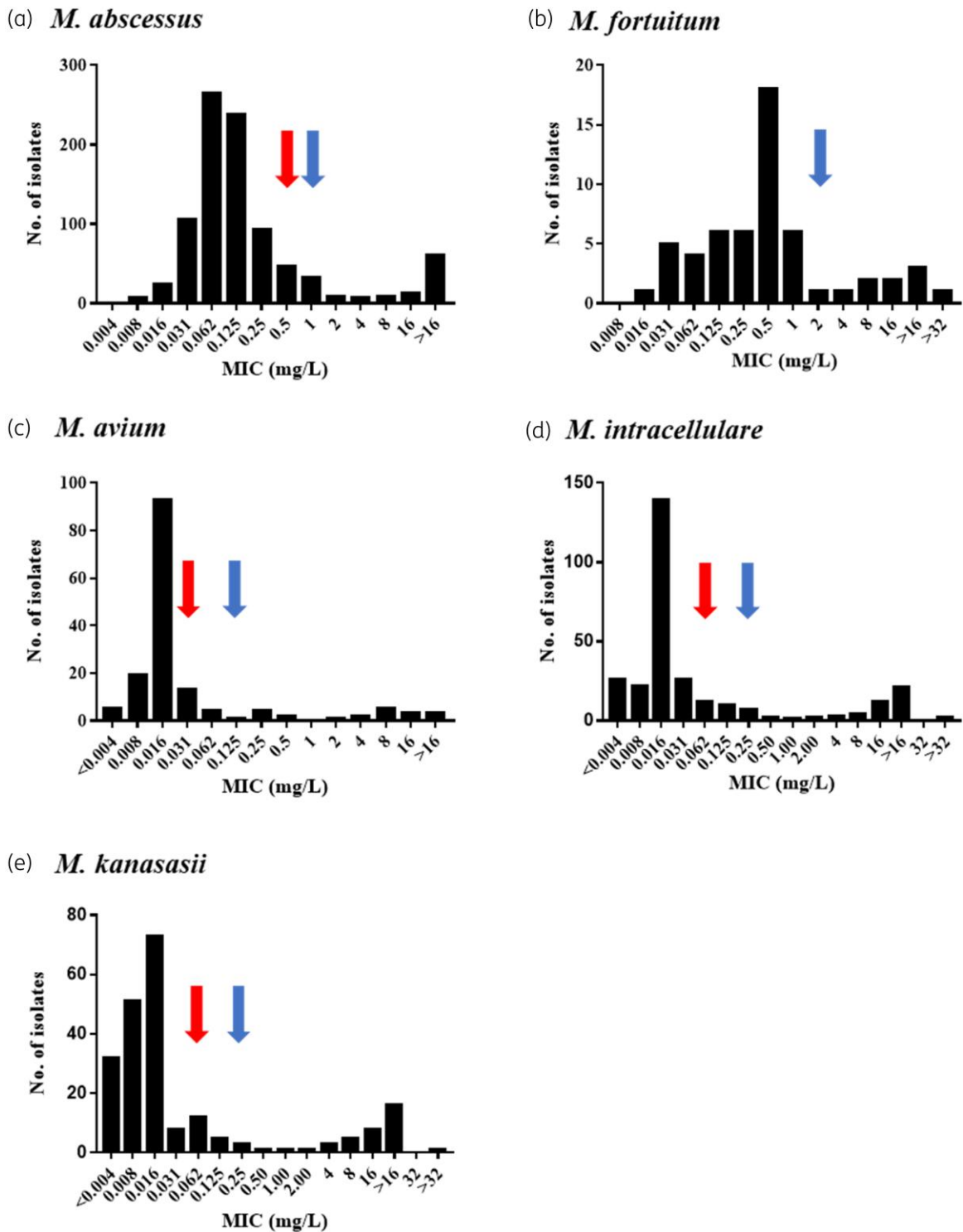


Figure 6. MIC distribution of the common NTM species in CAMHB. The blue arrow is pointing to the eyeball value and the red arrow is showing the T-ECOFF value. This figure appears in colour in the online version of JAC and in black and white in the print version of JAC.

to be certain of their quality. However, when we used ECOFFinder for the available data, T-ECOFF values differed by two 2-fold dilutions from the visual estimated values. Therefore, our findings

will remain uncertain and further studies are still required to define the ECOFF value for SGM and to corroborate our findings for *M. abscessus* species.

Table 4. Mutations associated with bedaquiline resistance in NTM species

Gene	Mutation in amino acid	Mutation in DNA	MIC (mg/L)	No. of isolates	Mycobacterium species	Type of isolate	Study (year)		
<i>atpE</i>	Ala35Ala	T105C	0.031	3	<i>M. abscessus</i>	Clinical	Pang et al. (2017) ⁴⁰		
			0.62	5					
			0.125	5					
			0.25	2					
			2	1					
	Ala63Met		4	1					
			>2	2	<i>M. flavescens</i>	Clinical	Aguilar-Ayala et al. (2017) ⁶		
			8	1	<i>M. novocastrense</i>	Clinical	Huitric et al. (2007) ³²		
			8	1	<i>M. shimoidei</i>	Clinical			
			4	1	<i>M. xenopi</i>	Clinical			
	Ala63Pro		8	1	<i>M. smegmatis</i>	Non-clinical	Segala et al. (2012) ⁴⁷		
			0.5	1	<i>M. smegmatis</i>	Non-clinical	Segala et al. (2012) ⁴⁷		
			16	1	<i>M. abscessus</i>	Non-clinical			
			16	1	<i>M. abscessus</i>	Non-clinical	Dupont et al. (2017) ²⁹		
			0.125	1					
Ala64Pro		0.004	1	<i>M. intracellulare</i>	Clinical on treatment	Alexander et al. (2017) ⁶²			
		2	1	<i>M. intracellulare</i>	Clinical post treatment				
		2	1	<i>M. intracellulare</i>	Clinical on treatment				
		>0.5	1	<i>M. smegmatis</i>	Non-clinical	Maeda et al. (2021) ³⁸			
		8	7	<i>M. abscessus</i>	Non-clinical	Segala et al. (2012) ⁴⁷			
Aps32Gly Asp28Ala Asp28Gly Asp28Val Asp776Asn & Asp32Ala & Gly31Gly Asp32Ala & Gly31Gly & Ser465Pro Asp29Val Asp32Val Glu61Asp Glu65Glu		T32C	4	9	<i>M. fortuitum</i>	Non-clinical			
			16	1	<i>M. smegmatis</i>	Non-clinical	Segala et al. (2012) ⁴⁷		
			>16	1	<i>M. smegmatis</i>	Non-clinical	Segala et al. (2012) ⁴⁷		
			16	1	<i>M. smegmatis</i>	Non-clinical	Maeda et al. (2021) ³⁸		
			0.19	1	G776A & T32G & G31A	Non-clinical	Dupont et al. (2017) ²⁹		
			0.25	1	T32G & G31A & T465C	Non-clinical			
			16	1					
			0.125	1					
			MIC ₉₀ =3	1			<i>M. smegmatis</i>	Non-clinical	Andries et al. (2014) ²⁴
			MIC ₉₀ =3	1					
4	1			<i>M. smegmatis</i>	Non-clinical	Segala et al. (2012) ⁴⁷			
0.062	2	A195G		<i>M. fortuitum</i>	Clinical	Pang et al. (2017) ⁴⁰			
0.25	1								
1	1								
16	1								

Continued

Table 4. Continued

Gene	Mutation in amino acid	Mutation in DNA	MIC (mg/L)	No. of isolates	Mycobacterium species	Type of isolate	Study (year)
Glu65Asp Gly24Gly		T65A	0.5	1	<i>M. smegmatis</i>	Non-clinical	Maeda et al. (2021) ³⁸
		T72C	0.062	6	<i>M. abscessus</i>	Clinical	Pang et al. (2017) ⁴⁰
Gly49Gly		C147T	0.12	8	<i>M. intracellulare</i>	Clinical	Pang et al. (2017) ⁴⁰
			0.25	4			
			0.5	6			
			>16	1			
			0.0156	6			
			0.031	2			
Gly62Gly		T186C	0.062	3	<i>M. kansasii</i>	Clinical	Pang et al. (2017) ⁴⁰
			0.12	2			
			1	1			
			4	1			
			>16	1			
			0.031	2			
			0.063	3			
			1	1			
			8	1			
			16	8			
Ile66Met			0.5	1	<i>M. smegmatis</i>	Non-clinical	Segala et al. (2012) ⁴⁷
			0.031	1			
			0.062	1			
			0.12	1			
Leu59Val Val62Val			>16	1	<i>M. smegmatis</i>	Non-clinical	Segala et al. (2012) ⁴⁷
			0.031	1			
			0.062	1			
			0.12	1			
Wild type			>16	1	<i>M. intracellulare</i>	Clinical	Alexander et al. (2017) ⁶²
			0.004–0.03 (0.025)	22			
mmpT5	No non-synonymous mutation was found		<0.003	1	<i>M. avium</i>	Clinical	Zweijpfenning et al. (2019) ⁶⁸
			0.0078–1	197	<i>M. abscessus</i>	Clinical	Li et al. (2018) ³⁴
			0.004	5	<i>M. intracellulare</i>	Clinical	Alexander et al. (2017) ⁶²
			0.008	1	<i>M. intracellulare</i>	Clinical	
			0.025	2	<i>M. intracellulare</i>	Clinical	
			0.004	1	<i>M. intracellulare</i>	Clinical	
			0.008	2			
			0.025	1			
			0.008	2			
			0.015	2			
			0.025	2			
			0.004	1			
			0.008	1			
0.008	1						
Ala23Pro & Glu177Lys Pro104FS		G67C & G529A C311_312insC	0.008	2	<i>M. intracellulare</i>	Clinical	
			0.015	2	<i>M. intracellulare</i>	Clinical	
			0.025	2			
			0.004	1			
Ala162Pro		G484C	0.004	1	<i>M. intracellulare</i>	Clinical	
			0.008	1			
Ilu19Ser & Glu177Lys Val35Gly & Glu177Lys Arg25Pro		T56G & G529A T104G & G529A G74C	0.008	1	<i>M. intracellulare</i>	Clinical	
			0.008	1	<i>M. intracellulare</i>	Clinical	
			0.004	1	<i>M. intracellulare</i>	Clinical	

MAB_4384	Ala152 Glu		0.062	2	M. abscessus	Clinical	Li et al. (2018) ³⁴
	Ala169 Ser		0.5	1	M. abscessus	Clinical	
	Del (MAB_4384 gene)	Del	0.0078	2	M. abscessus	Clinical	
			0.0156	3			
			0.031	18			
			0.062	39			
			0.125	36			
			0.25	11			
	Gln215 Arg		0.0078	1	M. abscessus	Clinical	
			0.031	1			
			0.062	1			
			0.125	4			
			0.5	1			
	Gly125 Asp & Gln215 Arg		0.062	1	M. abscessus	Clinical	
			0.125	2			
	His7 Arg & Glu142 Lys		0.125	1	M. abscessus	Clinical	
			0.25	4			
			0.5	1			
	N1T		0.062	2	M. abscessus	Clinical	
			0.125	1			
	WT		0.007	2	M. abscessus	Clinical	
			0.015	1			
			0.031	9			
			0.062	16			
			0.125	19			
			0.25	6			
			0.5	2			
			1	1			
	Trp88 Gly		0.062	2	M. abscessus	Clinical	
			0.125	1			
	Val31 Ile		0.031	1	M. abscessus	Clinical	
			0.125	2			
			0.25	1			
	Val31 IleI & Asp120 Asn			1	M. abscessus	Clinical	
	Val5 Met & His7 Arg & Glu142 Lys & Ala217 Ser			1	M. abscessus	Clinical	
MAB_2299c	Asp106fs	ins318A	2	1	M. abscessus	Non-clinical	Richard et al. (2018) ⁴³
	Glu181stop	C276	2	1			
	Gly215Ser	G541T	2	1			
	Leu151Pro	G643A	2	1			
	Leu40Trp	T452C	2	1			
	Pro92fs	T119G	2	1			
MAV_2152	Trp173Arg	A2544950G	0.06	1	M. avium	Clinical	Zweijpfenning et al. (2019) ⁶⁸
	Trp173Arg	A2544950G	0.125	1			Maeda et al. (2021) ³⁸
rpoB	Asp 776Asn		0.5	1	M. smegmatis	Non-clinical	
prpE	Ser465Pro		0.2	1	M. smegmatis	Non-clinical	

Association between genotype and phenotype bedaquiline resistance in NTM species

Genetic variants were identified in seven different genes: 22 mutations in *atpE*, 11 in *MAB_4384*, 9 in *mmpT5*, 6 in *MAB_2299c*, 1 in *MAV_2152*, 1 in *rpoB* and 1 in *prpE*. In addition, a study identified a new gene cluster called *MAB_1135c-MAB_1134c* that encodes a new MmpS-MmpL efflux pump system involved in the intrinsic resistance to bedaquiline and clofazimine in *M. abscessus*. *MAB_1135c-1134c* expression is also dependent on the *MAB_2299c* TetR repressor.^{6,24,29,32,34,38,43,47,52,62,68} Media used for antimicrobial susceptibility tests for these isolates varied across the studies. MIC values for the strains conferring resistance to bedaquiline also varied between 0.004 and >16.0 mg/L, with the highest MIC observed in isolates with mutations in the *atpE* gene. Mutations in the *atpE* gene occurred in both clinical and non-clinical isolates (Table 4).

The effect of bedaquiline in treating NTM species in animal models

Four studies evaluated bedaquiline only or bedaquiline-including regimens in treating skin infection (inoculation of footpad) in mice (44%; 4/9).^{9,55-57} Five studies reported on disseminated infection (56%; 5/9) in lung, liver and spleen.^{10,58-61} Mortality rate was reported in one study only.¹⁰ However, none of the studies that assessed bedaquiline-only efficacy in treating disseminated infections reported on follow-up, relapse, cure or relapse-free cure as treatment outcome.

Treatment outcome of bedaquiline-including regimens

Bedaquiline-including regimens decreased the footpad lesion index in mouse models of skin infection. A mean index of 3 (scale of 0–4) at Day 0 decreased to 1.8 and 1.42 after 4 and 8 weeks of treatment, respectively, in the bedaquiline+rifapentine-treated group and remained stable with no relapse reported.⁵⁵ Similarly, Converse et al.⁹ reported a decrease in lesion index to an average of 0.56 ± 0.25 and 0.3 ± 0.2 in all treatment regimens that included bedaquiline after 4 and 8 weeks of treatment, respectively. Komm et al.⁵⁷ also showed a decrease in the footpad lesions from a median of 2.0 to ≤ 1.0 within 1 week of bedaquiline+telacebec treatment for 1, 3 and 5 days in two different mouse models (BALB/c and SCID-beige).

A 2 week regimen of rifapentine/clofazimine/bedaquiline (RFP+CLF+BDQ), followed by 12 weeks follow-up resulted in a lesion index of 2.5 in 10% of treated mice. Additionally, a 4 week regimen of RFP+CLF+BDQ plus telacebec (RFP+CLF+BDQ+Q203) showed a lesion index of 1.4 in 10% of treated mice.⁹

Bedaquiline-including regimens also had the effect of decreasing bacterial burden (mean cfu) from 5.89 ± 0.22 at Day 0 to 1.095 ± 1.0 after 2 weeks of treatment⁹ and from 6.39 ± 0.30 to 0.19 ± 0.42 after 4 weeks of treatment⁵⁵ or negative (complete cure).⁹ Similarly, bacterial load declined from 6.62 ± 0.34 to culture negative within 5 days of initiating a bedaquiline+telacebec treatment regimen.⁵⁷

There was relapse in 15% (1.5/10 mice) with cfu counts ranging from 3.13 to approximately 5.08 log₁₀ after 2 weeks treatment and 12 weeks follow-up when treated with bedaquiline-including regimens.⁹ However, no cfu were detected in any other bedaquiline-containing regimen after 4 weeks of treatment and 21 weeks follow-up, suggesting a complete cure

of the treated mice using bedaquiline-including regimens.⁹ Only one (13%) study reported on death as a bedaquiline treatment outcome in 20% (2/10) of mice.¹⁰ None of the other studies included data on mortality.

These data suggested that the relapse rate is ~10%–15% in mice treated with a bedaquiline-containing regimen. However, more data are still required to have a concrete conclusion.

Treatment outcome of bedaquiline alone The efficacy of bedaquiline alone in treating infection caused by NTM species was evaluated only based on its ability to reduce bacterial burden. While five studies^{10,58-61} evaluated the efficacy of bedaquiline alone in treating NTM disseminated infection, only one study (13%) looked at its efficacy in treating NTM skin infection.⁵⁶ For this major outcome, meta-analysis was performed using six studies. To evaluate the pooled effect measure in animals (mice), we performed subgroup meta-analysis stratified by organ and by NTM species. The effect size was measured using SMD as reported in the methods. However, when using mean difference measures, the effect of bedaquiline remains significant with a slight decrease in the effect size.

Subgroup analysis by organ

Lung Subgroup meta-analysis in lungs showed that bedaquiline reduces the bacterial load (log₁₀ cfu) by 1.56 times in the bedaquiline treatment arm compared with no treatment, with low to moderate heterogeneity (SMD 1.56; 95% CI –2.35 to –0.77; $P < 0.0001$; $I^2 = 39\%$; 4 studies; 29 mice) (Figure 7, Analysis 1.1.1). To explore heterogeneity, the Obregón-Henao 2015 study was excluded from the analysis, resulting in decreased heterogeneity of $I^2 = 20\%$. However, the effect measure remained significant (SMD –1.26; 95% CI –2.06 to –0.46; $P < 0.0001$; $I^2 = 20\%$).

Liver There was no evidence of a difference between bedaquiline treatment and no treatment (SMD –2.51; 95% CI –5.82 to 0.80; $P = 0.14$; 2 studies; 20 mice) (Figure 7, Analysis 1.1.2). However, there was substantial heterogeneity among the two studies that examined the efficacy of bedaquiline in reducing the bacterial load in the liver ($I^2 = 91\%$; $P = 0.0007$).

Spleen A forest plot showed a significant difference in reducing the bacterial load in the spleen between bedaquiline treatment and no treatment with high heterogeneity (SMD –1.49; 95% CI –2.59 to –0.38; $P = 0.008$; $I^2 = 71\%$; 5 studies; 34 mice) (Figure 7, Analysis 1.1.3). When the Lounis et al.⁵⁹ study was excluded from the analysis, the heterogeneity decreased from high to moderate ($I^2 = 37\%$; $P = 0.19$) and the effect measure remained significant (SMD –1.95; 95% CI –2.80 to –1.10; $P < 0.00001$) (Figure S5). However, when the Lounis et al.⁵⁹ and Sarathy et al.⁶¹ studies were excluded from the analysis, no evidence of heterogeneity was detected and the effect measure remained significant (SMD –2.34; 95% CI –3.11 to –1.58; $P < 0.00001$; data not shown).

Skin One study (1/3) compared bedaquiline alone versus a control group and provided data for up to 14, 30 and 60 days⁵⁶ was included in the meta-analysis. A forest plot showed a significant difference in the reduction in cfu between the treatment

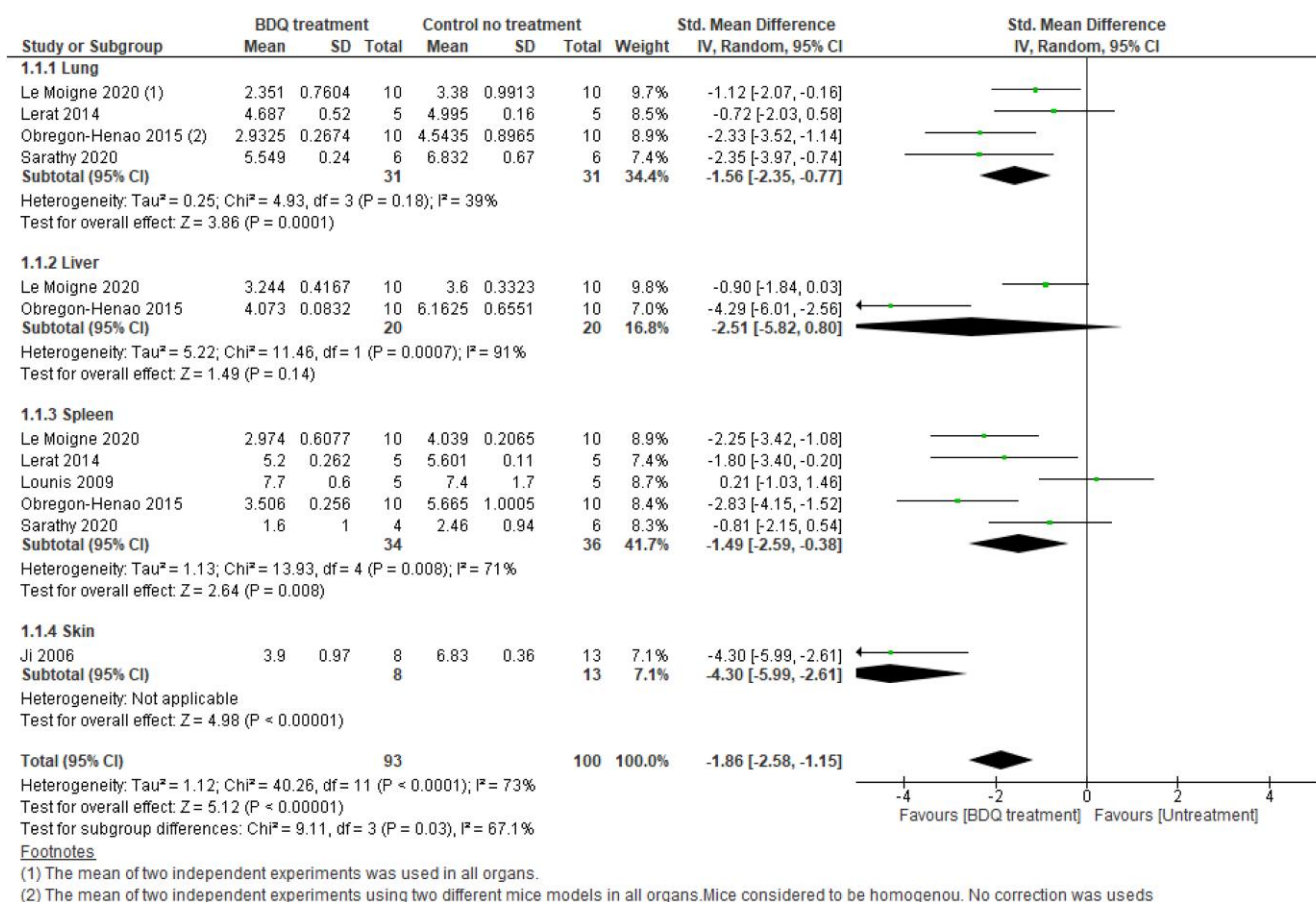


Figure 7. Forest plot of bedaquiline (BDQ)-treated animals versus control (no treatment) animals. Outcome 1.1: BDQ effect on bacterial load for each group up to 30 days (subgroup by organs). The effect of BDQ on bacterial load in each group was expressed as log₁₀ cfu ± SD. This figure appears in colour in the online version of JAC and in black and white in the print version of JAC.

and control arms (SMD -4.30; 95% CI -5.99 to -2.61; P < 0.00001; one study, 8 mice, heterogeneity is not applicable) (Figure 7, Analysis 1.1.4).

Overall, the effect of bedaquiline, stratified by organ (lung, liver, spleen and skin) revealed a significant reduction in bacterial load in lung, spleen and skin but not liver within 30 days. Bedaquiline reduced the bacterial load on average by 1.86 times in the bedaquiline treatment arm compared with the no-treatment arm (SMD -1.86; 95% CI -2.58 to -1.15; P < 0.0001; I² = 73%) (Figure 7). The overall heterogeneity between studies was substantial (I² = 73%). Similarly, subgroup heterogeneity tests showed a significant difference between organs (P = 0.03; I² = 67%). This is largely due to the heterogeneity between studies, particularly Ji's.⁵⁶ However, when a susceptibility test was performed and the study by Ji was removed, no significant difference in heterogeneity between the three organs (lung, liver and spleen) was detected (Figure S6).

As a part of sensitivity analysis, we conducted the meta-analysis by including data up to 60 days from two studies (Lerat *et al.*¹⁰ and Lounis *et al.*⁵⁹). The overall pooled effect measure remains significant with an increase in the effect of bedaquiline, resulting in reduction of the bacterial load on average

by 2.29 (SMD -2.29; 95% CI -2.97 to -1.62; P < 0.00001) (Figure S7).

Collectively, this data indicates that bedaquiline can significantly reduce bacterial burden, and it is more effective in the lung and spleen than in the liver. However, depending on the bacterial species and strain, a longer treatment period might be required. Due to the small sample size further studies are still required to confirm this data.

Subgroup analysis by NTM species We then looked at the effect of bedaquiline stratified by NTM species (*M. abscessus*, *M. avium* and *M. ulcerans*). Since one study⁵⁹ showed a bedaquiline effect against *M. avium* species in spleen only, we were only able to perform meta-analysis of bedaquiline treatment efficacy in eradicating bacterial burden in one organ (spleen).

Subgroup analysis by NTM species in spleen

M. abscessus Subgroup meta-analysis showed that bedaquiline reduces the log₁₀ cfu of *M. abscessus* significantly by 1.95 times. However, there was moderate heterogeneity between these studies (SMD -1.95; 95% CI -2.80 to -1.10; P < 0.00001; I² = 37%) (Figure 8, Analysis 1.2.1). Excluding data from Sarathy

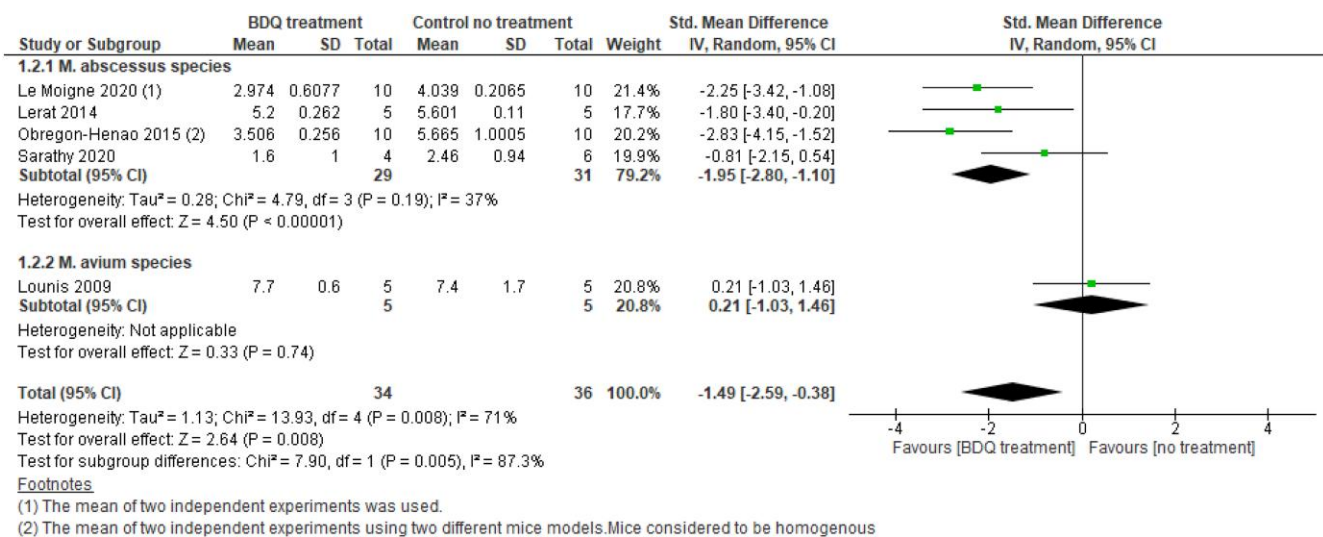


Figure 8. Forest plot of bedaquiline (BDQ)-treated animals versus control (no treatment) animals. Outcome 1.2: BDQ effect on bacterial load up to 30 days (subgroup by NTM species). The effect of BDQ on bacterial load was expressed as \log_{10} cfu \pm SD. This figure appears in colour in the online version of JAC and in black and white in the print version of JAC.

et al.⁶¹ study, decreased heterogeneity ($I^2=0\%$) within this subgroup (SMD -2.34 ; 95% CI -3.11 to -1.58 ; $P<0.00001$) (Figure S8, Analysis 1.2.1).

M. avium One study⁵⁹ assessed the effect of bedaquiline against *M. avium* in the spleen for up to 30 days. There was no difference between bedaquiline treated or untreated arms (SMD -0.21 , 95% CI -1.03 to 1.46 , $P=0.74$) (Figure 8, Analysis 1.2.2).

Overall, within 30 days, stratification by NTM species revealed a significant effect of bedaquiline in reducing the bacterial load of the two NTM species: *M. abscessus* and *M. avium* by 1.49 (SMD -1.49 ; 95% CI -2.59 to -0.38 ; $P=0.008$). However, the overall heterogeneity between studies was high ($I^2=71\%$; $P=0.008$) (Figure 8, Analysis 1.2) with substantial heterogeneity between the subgroups ($I^2=87.3\%$; $P=0.005$).

However, when data of 60 days from two studies (Lerat et al.¹⁰ and Lounis et al.⁵⁹) were used in the meta-analysis, the overall bedaquiline efficacy in reducing the bacterial load was increased from 1.49 to 2.54 (SMD -2.54 ; 95% CI -3.23 to -1.86 ; $P<0.00001$) (Figure S9, Analysis 1.2) with a significant change in the effect on *M. avium* species treatment (SMD -4.49 ; 95% CI -7.31 to -1.68 ; $P=0.002$) (Figure S9, Analysis 1.2.2). The overall heterogeneity between subgroups, however, reduced from high to moderate ($I^2=48.8\%$).

Longer treatment duration with bedaquiline may be required to show a significant effect on NTM species, particularly on *M. avium* species. However, due to the small sample size, the evidence is uncertain.

The effect of bedaquiline in treating NTM species in humans

Six (30%; 6/20) cases were successfully cured (symptom improvement and culture conversion) with no relapse reported when a bedaquiline-including regimen was used.^{63–67} All of these cases had NTM extrapulmonary infection caused by either *M.*

abscessus, *M. fortuitum*, *M. avium* or *Mycobacterium marinum* (Table 3), in which one case had cutaneous infection in the leg and four patients had disseminated NTM infection. Three were immunocompromised with HIV coinfection, and one was a child with AML complicated by calcaneal osteomyelitis. Treatment duration varied between 3.5 and 18 months. Although some adverse events such as nausea⁶⁶ and a mild QTc prolongation in one case⁶³ were reported, bedaquiline was well tolerated.

However, data from the remaining 14 patients with NTM pulmonary diseases fluctuated between symptom improvement and culture conversion¹¹ to a treatment failure.⁶⁸ Ten of these patients that had potentially life-threatening NTM disease and were failing standard therapy: off-label bedaquiline was added to their current antibiotic regimens as a salvage therapy.¹¹ Various outcomes were observed throughout the study. However, none of these patients had three consecutive negative cultures or were reported to be cured completely, despite the improvement in semi-quantitative sputum culture scores, symptoms and radiographic data, which is not included in our review. Four of the 10 patients, along with 3 other patients who experienced relapse, were reported to have relapsed, despite a positive initial response to the treatment, in the Alexander et al.⁶² trial. This case series was focused on the genotypic and phenotypic characteristics of the samples from the relapse patients only and did not report on details of the clinical or microbiological data during treatment. Thus, we were unable to conclude treatment outcome from this case series.⁶² The relapse was associated with a 2- to 8-fold (from 0.004 to 0.03 mg/L) increase in MICs of bedaquiline and in one case the increase in MIC was very high (from 0.004 to 2.0 mg/L). Genetic analysis of the relapse strains from all patients revealed that these strains had non-synonymous mutations in the *mmpT5* and *atpE* genes, in which the *atpE* variant (Ala65Pro) was reported in only two cases. Moreover, in one patient with MAC lung infection, culture conversion was not achieved upon treatment with a

Table 5. Summary of findings: human studies treatment outcome

Study	Disease	Species	Treatment duration (months) with BDQ	Treatment outcome			
				Culture conversion	Cure	Relapse	Symptom improvement
Alexander <i>et al.</i> (2017) ⁶²	Pulmonary	<i>M. avium</i> complex and <i>M. intracellulare</i>	≥6	0/3	0/3	3/3	0/3
Chan <i>et al.</i> (2021) ⁶³	Extrapulmonary	<i>M. abscessus</i>	8	1/1	1/1	0/1	1/1
Erber <i>et al.</i> (2020) ⁶⁴	Extrapulmonary	<i>M. fortuitum</i> complex	3.5	1/1	1/1	0/1	1/1
Gil <i>et al.</i> (2021) ⁶⁵	Extrapulmonary disease	<i>M. abscessus</i> and <i>M. avium</i>	21,14	2/2	2/2	0/2	2/2
Meybeck <i>et al.</i> (2021) ⁶⁶	A mix between extrapulmonary and pulmonary	<i>M. marinum</i>	12	1/1	1/1	0/1	1/1
Pearson <i>et al.</i> (2020) ⁶⁷	Extrapulmonary	<i>M. abscessus</i>	> 3.5	1/1	1/1	0/1	1/1
Philly <i>et al.</i> (2015) ¹¹	Pulmonary	<i>M. avium</i> complex and <i>M. intracellulare</i>	6	0/10	0/10	4/10	4/10
Zweijpenning <i>et al.</i> (2019) ⁶⁸	Pulmonary	<i>M. avium</i> complex	≥6	0/1	0/1	N/A	0/1

N/A, not applicable.

bedaquiline-including regimen. In fact, the patient experienced treatment failure due to the emergence of bedaquiline resistance caused by a mutation in MAV_2152 locus Trp173Arg. The resistant isolate had an increase in bedaquiline MIC (from 0.03 to 0.06 and 0.125 mg/L), within 6 and 12 months of treatment, respectively.⁶⁸ However, despite the unfavourable treatment outcome, bedaquiline was well tolerated and there were no major side effects reported in all cases with NTM pulmonary diseases. A summary of the treatment outcomes can be found in Table 5.

Discussion

Managing the treatment of NTM infections is usually challenging due to the intrinsic resistance of these bacteria to most of the available antibiotics, including some anti-TB drugs.⁷¹ Although bedaquiline is an effective therapy for drug-resistant TB, there is little information on its antibacterial efficacy against NTM species *in vitro* or *in vivo*. Therefore, we aimed to consolidate all available evidence in the literature regarding bedaquiline activity against various NTM species and evaluate the MIC distribution *in vitro* and its efficacy *in vivo* (in animal models and human studies) while scrutinizing the methodological approaches of different studies.

The effect of bedaquiline in treating NTM species in *in vitro* studies

For most bacterial species, the concept of using MIC distributions among several other tools for determining clinical breakpoints has been extensively adopted by EUCAST.⁷² Understanding the distribution of MICs throughout the circulating population of mycobacterial isolates is required for DST.⁷³ MIC distributions might fluctuate depending on the antimicrobial susceptibility testing method used. Although bedaquiline MICs of >2000 NTM

isolates had been reported using eight different platforms/media, MIC distribution data were insufficient in most of these platforms for most of the NTM species. However, we were able to evaluate and compare the WT MIC distribution of RGM and SGM in CAMHB and determine the ECOFF value for *M. abscessus* in CAMHB only.

Our study revealed that bedaquiline has a potential *in vitro* inhibitory activity against the most common clinically relevant species of RGM (*M. abscessus* and *M. fortuitum*) as well as SGM (NTM) species (*M. avium*, *M. intracellulare* and *M. kansasii*), with a higher potency against SGM (NTM) species than RGM. Using CAMHB, the MIC distribution range was wide (≤ 0.008 to ≥ 32 mg/mL) for RGM and SGM. While the MICs for the majority of clinically relevant SGM (NTM) species ranged between 0.008–0.031 mg/L, bedaquiline MICs for the majority of RGM isolates (*M. abscessus* and *M. fortuitum*) were higher and were in the range 0.031–0.25 mg/L and 0.031–1.0 mg/L, respectively. However, as the number of SGM was insufficient, the finding that SGM seem to be more susceptible to bedaquiline *in vitro* than RGM species requires further investigation.

Thus far, there is no bedaquiline MIC cut-off value for NTM species. Due to the lack of a standardized method with a defined MIC concentration range and cut-off value specific for bedaquiline susceptibility testing in NTM species, the cut-off or ECOFF value used by various studies was variable. Additionally, studies varied in the control strains used, type of data reported, and MIC range tested. The inconsistent data made it difficult to set proper ECOFFs. These variations, with the rise in the incidence in NTM infection, highlight the necessity for a standardized method with a properly defined range, and quality control measures specific to bedaquiline susceptibility testing for NTM species. Discrepancies in MIC cut-off values for MIC determination in different platforms were reported in *M. tuberculosis* complex as well.⁷⁴ Considerable effort has been made by the WHO and EUCAST to establish a standardized method and cut-off values for MIC testing in

M. tuberculosis for various antibiotics, including bedaquiline, but little has been done for NTM species.

In this review, we compiled and analysed bedaquiline MIC data from various studies based on MIC methods used, to obtain a more accurate and useful ECOFF value. Despite the challenges in compiling these data, we were able to propose a T-ECOFF value of 0.5 mg/mL for *M. abscessus* in CAMHB from three acceptable distributions of over 361 isolates from different laboratories across the world. This value was concomitant with the cut-off value proposed by Chew,²⁷ when 211 *M. abscessus* isolates were evaluated using Sensititre plates.²⁷ However, we should be careful when comparing cut-off values from different methods. The MIC distribution and the visual cut-off value of 0.125 mg/L were similar between SGM species using CAMHB. Due to the insufficient data with truncated/skewed distributions, the ECOFF value of the SGM species could not be confirmed statistically.

In general, the MIC distribution of NTM species was similar to that of the bedaquiline MIC distribution of *M. tuberculosis* species, especially when using the Middlebrook 7H9 broth microdilution by Ismail et al.⁷⁵ Critical concentrations of 0.25 and 1.0 mg/L when using 7H11 and MGIT media, respectively, for *M. tuberculosis* was recommended by the WHO. However, when focusing on *M. tuberculosis* and SGM MIC distributions and cut-off values, it appears that SGM species are more susceptible to bedaquiline than *M. tuberculosis* species, implying that bedaquiline is more effective against SGM than *M. tuberculosis*. Of course, caution must be applied when comparing cut-off values derived using fundamentally different growth media and MIC methods.

Association between genotypic and phenotypic bedaquiline resistance in NTM species

We have generated the most comprehensive dataset of the genotypic mutations associated with bedaquiline phenotype (susceptibility and resistance) in NTM species to date. However, in the absence of a definite cut-off value in different MIC platforms and due to inadequate data, it was difficult to statistically evaluate the association between mutations in various genes and phenotypic resistance to bedaquiline in each NTM species.

Only a single mutation (*atpE* Ala63Met) is likely to be clinically relevant. This mutation was reported in both RGM (2 *Mycobacterium flavescens* and 1 *Mycobacterium novocastrense*) and SGM (2 *Mycobacterium xenopi* and 1 *Mycobacterium shimoidei*)³² and these isolates were found to be naturally resistant to bedaquiline with MICs of 4–8 mg/L. Since the Ala63 residue was conserved in many mycobacterial species, this mutation in the *atpE* gene could be of clinical importance and can be used as a marker of bedaquiline resistance.

Based on our proposed T-ECOFF value (0.5 mg/L) of *M. abscessus* when utilizing CAMHB, none of the mutations described in clinical isolates could be associated with bedaquiline resistance. However, non-clinical isolates with mutations in the MAB 2299c gene had an MIC of 2.0 mg/mL, suggesting that these mutations could be a relevant resistance marker to consider when treating *M. abscessus* infections with bedaquiline and clofazimine in clinical settings.⁴³

While the proposed eyeball cut-off value of 0.125 mg/L for SGM (NTM) revealed no mutations associated with bedaquiline resistance, the 0.031 mg/L cut-off value revealed that a mutation (2544950A>G; Trp173Arg) in the MAV 2152 gene of clinical isolates of *M. avium* could be associated with bedaquiline resistance. This mutation was found in a patient who developed *in vivo* resistance to bedaquiline within 6 to 12 months of commencing a bedaquiline-containing regimen and had MICs of 0.06 and 0.125 mg/L, which were 20- to 40-fold higher than for the WT isolate.⁵⁸ Thus, this mutation may have therapeutic significance and could be used as a bedaquiline resistance marker. Although mutations in the *mmpT5* gene were found in patients who relapsed after ≥ 3 months of bedaquiline-including therapy, MICs for most variants were within the *in vitro* bedaquiline susceptible range, except for the *mmpT5* T137G (Val46Gly) mutation. This mutation conferred an MIC of 0.03 mg/mL, which was 8-fold higher than for the WT, indicating that this mutation may have clinical implications. However, the *in vitro* data do not always reflect the *in vivo* resistance, and further studies are still required to determine the clinical critical concentration for bedaquiline in various NTM species. Unfortunately, because all the above-mentioned mutations were only reported once in clinical isolates, this knowledge might not have a significant impact on clinical practice. Thus, further research is still required to identify the relevance of these mutations in bedaquiline resistance in NTM species.

The effect of bedaquiline in treating NTM species in animal models

In the animal model studies included in this review, bedaquiline *in vivo* activity was evaluated against three NTM species only: *M. ulcerans*, *M. avium* and *M. abscessus* in skin, lung, spleen and liver. Bedaquiline alone showed an effect against *M. ulcerans* in reducing the lesion index in skin infection after treatment for 8 weeks.⁵⁶

Overall, our meta-analysis revealed that bedaquiline alone had a significant effect in reducing the log cfu outcome in major organs, except the liver, compared with the control group. The lack of a significant effect in the liver may be explained by the variations between studies, including mouse strain characteristics, bacterial strains with different MICs, and other methodological variations.

Bedaquiline showed a higher effect on *M. avium* species than on *M. abscessus* only after 60 days of treatment in the spleen. Despite the limited data, this finding may imply that a short bedaquiline treatment is sufficient to confer a significant effect against *M. abscessus* infection, whereas a longer bedaquiline treatment duration may be required for *M. avium* species. This is in line with the difficulty of treating *M. avium* species in humans but contradicts SGM species' *in vitro* susceptibility to bedaquiline. However, in humans, a longer treatment period for at least 1 year was recommended for *M. abscessus* infection as well.⁷⁶ Due to insufficient data, we could not perform subgroup analysis on the effect of bedaquiline on NTM species in other organs (lung and liver).

Bedaquiline combined with various antibiotics (rifampicin or rifapentine and/or Q203 or clofazimine) showed high activity against *M. ulcerans* and significantly reduced the bacterial

burden in skin infection^{9,55-57} with a complete cure without relapse after 2 weeks of treatment.⁹ The high activity of the BDQ+Q203+CLF regimen could be due to the fact that these drugs are targeting and disrupting the electron transport chain (ETC) and oxidative phosphorylation in a similar mechanism to what was found in *M. tuberculosis*.^{77,78} Similarly, bedaquiline in combination with clarithromycin and/or amikacin reduced the bacterial burden in the spleen against *M. avium* infection after 3 months of treatment. The effect of this regimen was found to be better than bedaquiline alone.⁵⁹ Moreover, bedaquiline combined with either imipenem or clofazimine also showed a significant effect in reducing the bacterial burden against *M. abscessus* species in the major organs.^{58,60} These drugs have been shown to be extremely effective against both drug-susceptible and drug-resistant *M. tuberculosis* isolates in animal models and human studies.⁷⁹⁻⁸¹ Despite the cross-resistance between bedaquiline and clofazimine, and other downsides observed in *M. tuberculosis*,⁸² this combination treatment showed a promising effect against *M. abscessus* species as well.

The time required for bedaquiline to significantly reduce bacterial burden varied between the studies: Obregón-Henao *et al.*⁶⁰ showed that bedaquiline reduced bacterial burden after 5 days, Sarathy *et al.*⁶¹ showed a significant effect of bedaquiline within 11 days and Lerat *et al.*¹⁰ showed that bedaquiline had a significant effect only after 2 months of treatment. As mentioned previously, these variations could be due to disparities in the bacterial strain, drug dose, animal model, disease induction protocols, and the analysis approach used in these studies. Although most of the studies evaluating bedaquiline in treating disseminated infection used *M. abscessus* species, the strain and its *in vitro* MIC used varied across the studies. Additionally, the bedaquiline dose varied across the studies (Table 3).

Animal models for evaluating antibiotic activity against NTM species infection also varied between studies and included both immunocompetent and immunodeficient mouse strains. Bedaquiline showed a significant effect in decreasing bacterial load in the liver of infected C3HeB/FeJ mice within 25 days in one experiment but not in another experiment of the same strain when using a different infection route.⁵⁸ Studies that used γ -IFN knockout (GKO) mice demonstrated a substantial effect of bedaquiline treatment in lowering bacterial loads,⁶⁰ but work in nude mice (athymic mice with depletion of T-cells) failed to show a reduction in bacterial burden and mortality prevention in the first month of treatment and required longer treatment to show an effective reduction in the bacterial load.¹⁰ Obregón-Henao *et al.*⁶⁰ showed that most mouse models with significant innate or acquired immune deficiencies are nonetheless able to remove a high degree of *M. abscessus* infection.⁶⁰ The susceptibility of mouse strains to infection with virulent *M. tuberculosis* also varied, depending on the genotype, with some strains being more resistant than others, which could affect treatment outcomes.^{83,84} A C3HeB/FeJ mouse model shows more necrotic granulomas in the lungs after *M. tuberculosis* infection, which allows bacteria to replicate, and fewer tubercular granulomas in the spleens and livers, resulting in greater bacterial clearance in the liver and spleen.⁸⁵ These discrepancies in mouse strain susceptibilities emphasize the importance of developing a suitable NTM infection model that provides a high, constant and reproducible bacterial load in organs for therapeutic trials. However, it is

important to keep in mind that no animal model can accurately depict the entire clinical condition. Thus, the outcomes of inappropriately designed animal model research may yield indirect results.⁸⁶

Collectively, bedaquiline alone or bedaquiline-including regimens demonstrated high activity against NTM species in various mouse models. Although the evidence is uncertain due to the limited data, the significant *in vivo* activity of bedaquiline shown in this review and other studies dictates the need for further research in clinical settings with special attention to the possible cross-resistance when using bedaquiline and clofazimine in combination.

The effect of bedaquiline in treating NTM species in humans

Case-series studies have many limitations, such as restricting researchers from generalizing results, or proving cause-and-effect relationships, with a risk of overinterpretation and publishing bias. Despite these limitations, case reporting has many advantages: the ability to identify novelties and present rare, unusual, uncontrollable observations about symptoms, clinical findings, disease progression, intervention complications, drug side effects, and so on.⁸⁷ Systematic reviews of observational studies can still be effective to summarize findings and compare case series, as well as identifying methodological concerns.⁸⁸

Most of the successfully cured cases had NTM-related extrapulmonary diseases with NTM disseminated infection with either HIV coinfection or leukaemia. Despite the severity of the diseases in these cases, bedaquiline proved to be an effective therapeutic option in the treatment of extrapulmonary diseases caused by diverse NTM species. Bedaquiline was used in combination with various antibiotics: levofloxacin, moxifloxacin or tedizolid, and clofazimine, against *M. fortuitum* complex, *M. marinum* and *M. avium*, respectively, or in combination with either omadacycline or clofazimine, against *M. abscessus* species. In cases of immunocompetent patients, bedaquiline was used in a combination of >2 drugs.

The successful use of bedaquiline-including regimens (Table 6) could be due to the intrinsic activity of bedaquiline. However, we cannot exclude the efficacy of other drugs. In a combination therapy, it is impractical to assign the positive outcomes to a single drug. Bedaquiline in combination with a highly active fluoroquinolone (levofloxacin), which inhibits bacterial DNA replication, to effectively suppress mycobacterial growth could be the essential element of the successful treatment of *M. fortuitum* complex.⁶⁴ Omadacycline also showed great efficacy *in vitro* against *M. abscessus* species, as well as a wide range of Gram-positive and Gram-negative bacteria,⁸⁹ through its mechanism of binding to the bacterial ribosome and inhibiting protein synthesis.⁹⁰ Recently, omadacycline was approved by the FDA for the treatment of skin and skin structure infections, as well as community-acquired pneumonia, after Phase 3 clinical trials revealed good efficacy.⁹¹ Moreover, the present cases revealed a high *in vivo* efficacy of bedaquiline/clofazimine combination against *M. abscessus* (RGM) and *M. avium* (SGM), which correlated with the *in vitro* synergistic effect of these drugs against NTM species as reported by Ruth *et al.*⁴⁵ Bedaquiline/clofazimine combination has also proved to be effective against both drug-

Table 6. Summary of findings: effective bedaquiline-including regimens

In animal model studies		In human studies	
Regimen	<i>Mycobacterium</i> species	Regimen	Species
RIF or RFP and/or Q203 or CLF	<i>M. ulcerans</i>	BDQ + LVX	<i>M. fortuitum</i>
CLR and/or AMK	<i>M. avium</i>	BDQ + OMC or CLF	<i>M. abscessus</i>
BDQ combined with either IPM or CLF	<i>M. abscessus</i>	BDQ + MFX	<i>M. marinum</i>
		BDQ + TDZ and CLF	<i>M. avium</i>

RIF, rifampicin; AMK, amikacin; BDQ, bedaquiline; CLF, clofazimine; CLR, clarithromycin; IPM, imipenem; RFP, rifapentine; Q203, telacebec; MFX, moxifloxacin; OMC, omadacycline; TDZ, tedizolid; LVX, levofloxacin.

susceptible and drug-resistant *M. tuberculosis* isolates.^{79–81} It has been shown that bedaquiline reduces the time to sputum culture negativity and enhances outcomes in MDR *M. tuberculosis* patients.^{65,92} Due to the intrinsic and acquired resistance, NTM species are posing a significant challenge to treat and thus require a prolonged combination therapy, especially for pulmonary diseases.⁹³ The exact time for culture conversion was not reported clearly in human studies, so we could not estimate the time for culture conversion of cured NTM cases. However, the duration required to complete treatment in successful cases varied between 3.5 and 18 months with an average of 9.4 months. Considering the severity of disseminated infection, the treatment regimens with the reported durations in these cases could be a promising therapy for NTM extrapulmonary disease and is worth further study in a larger cohort. Nevertheless, the emergence of bedaquiline resistance should be evaluated carefully.

In the cured cases, bedaquiline MICs for the RGM isolates varied between 0.12 and <0.0625 mg/L for *M. abscessus* and were 0.015 mg/L for *M. fortuitum*. Despite the possible variations in MIC platforms, these values are within the susceptible range for bedaquiline proposed in this review and other studies.^{6,45} Unfortunately, *in vitro* bedaquiline MIC data for the slow-growing isolates of *M. avium* and *M. marinum* were not provided. In the case of *M. marinum* infections, routine susceptibility testing has not yet been recommended. *M. marinum* was found to be susceptible to moxifloxacin (MIC₉₀) at 1 to 2 mg/L. Little is known about the relationship between *M. marinum in vitro* susceptibilities and clinical response.⁹⁴ In the absence of MIC data for these isolates (*M. avium* and *M. marinum*) from clinical cases, it was difficult to identify the correlation between *in vitro* susceptibilities and clinical response.

In the cases of NTM pulmonary disease, treatment with a bedaquiline-including regimen (Table 3) did not lead to a favourable clinical outcome with a complete cure, despite the initial response in many cases.^{11,62,68} However, most of these cases had advanced pulmonary disease and had been taking complementary medications for a long time, with little or no response.^{11,68} Bedaquiline was used as a salvage treatment option in many cases.

Several factors could be contributing to the unfavourable outcome. A major factor seems to be the emergence of resistance to bedaquiline within 3–8 months, which resulted in a 2- to 8-fold increase in *in vitro* MIC of bedaquiline. WGS showed that the resistant isolates of MAC contained non-synonymous mutations in the *mmpT5* and *atpE* genes, with *atpE* variations (Ala65Pro)

occurring in just two cases,⁶² and a W173R mutation in the MAV_2512 locus in one case in the study by Zweijpenning et al.⁶⁸ These mutations were discussed elsewhere in the review. However, both *mmpT5* and MAV_2512 loci encode a transcriptional regulator of the TetR family that regulates the *mmpS5-mmpL5* operon in MAC species, which is equivalent to the *rv0678* gene in *M. tuberculosis*. It was shown previously that mutations in the *rv0678* gene cause resistance to bedaquiline and cross-resistance to clofazimine in *M. tuberculosis*.⁸² A limitation of these studies is that none of them investigated the MIC data or the emergence of resistance in *M. abscessus* isolates. It should be underlined that for *M. tuberculosis*, the WHO advises performing phenotypic DST at the very least when treatment is initiated.⁹⁵

Another possible contributing factor to unfavourable outcomes could be drug–drug interactions and suboptimal drug regimens during coadministration of bedaquiline with other drugs. For example, a combination of rifamycins and bedaquiline, which was used in these studies, is not recommended. Rifamycins are known to activate the CYP450 system pathway, which is responsible for bedaquiline metabolism. Considering that bedaquiline is mostly metabolized by CYP3A, there is a risk of drug interactions when it is combined with CYP3A inducers or inhibitors. According to the FDA, combination of bedaquiline with any strong CYP3A4 inducers should be avoided.^{96,97} However, according to the included *in vitro* studies, a bedaquiline and rifabutin combination showed a high bactericidal effect of bedaquiline against *M. abscessus* and this was boosted when amikacin was added.⁹⁸ Similarly, bedaquiline in combination with rifampicin showed a significant effect on decreasing bacterial burden of *M. abscessus* ATCC 19977 in a mouse model.^{10,55,56} Despite the importance of *in vitro* and *in vivo* animal models, they may not always accurately reflect the human system, especially in the case of NTM pulmonary disease.^{93,99} Moreover, drug–drug interaction between bedaquiline and rifabutin in the presence of other drugs is still unclear and requires further investigation.

WT MAC (which include *M. avium* isolates) as well as *M. abscessus* are known to be susceptible to macrolide antibiotics. However, resistance to macrolide therapy, either alone or in combination, could occur within a few months.¹⁰⁰ A regimen that includes bedaquiline may be an effective alternative against both *M. avium* and *M. abscessus*.

However, treatment outcome of patients with macrolide-resistant MAC pulmonary disease (MR-MAC-PD)¹⁰¹ or *M. abscessus* infections, with isolates having inducible resistance to

macrolides, were found to be poor.^{102,103} Eight of the 10 cases reported by Philley *et al.*¹¹ had macrolide-resistant isolates and the fact that bedaquiline was added to already failing regimens, with some cases having macrolide-resistant isolates, may have contributed to the poor outcome of the bedaquiline-including regimens. Interestingly, inducible macrolide resistance after exposure to clarithromycin has been described in *M. abscessus* subsp. *abscessus* infection but not in *M. abscessus* subsp. *massiliense* infection,^{102,104} suggesting differential response at subspecies level, which highlights the importance of performing speciation at subspecies level in clinical studies.

NTM-related diseases are known to be difficult to treat, with various therapeutic options available with poor treatment outcome. Due to the small sample size of the included studies, the question of the review regarding the efficacy of bedaquiline-including regimens in the treatment of NTM pulmonary disease could not be answered precisely. However, due to bedaquiline's bactericidal activity shown in *in vitro* studies, as well as animal model studies, against various mycobacterial species and its high efficacy in NTM extrapulmonary diseases, bedaquiline may still be a potential treatment option for NTM pulmonary disease. Thus, further studies are still required to evaluate the efficacy of bedaquiline alone or in combination with other drugs in treating NTM pulmonary disease in a randomized clinical setting with a well-optimized regimen and different stages of the disease.

Limitations and strengths

A comprehensive assessment of bedaquiline efficacy from 43 studies *in vitro* and *in vivo* (animal models and human) from across the world was performed. We proposed the bedaquiline cut-off value for five of the clinically most important NTM species: two RGM (*M. abscessus* and *M. fortuitum*) and three SGM (NTM) species (*M. avium*, *M. intracellulare* and *M. kansasii*) in CAMHB. We generated a profile of mutations that could be associated with bedaquiline resistance. Furthermore, we performed meta-analysis for the bedaquiline effect on animal models and evaluated the treatment outcome in humans. However, and despite the strengths of our study, several limitations were encountered.

In vitro studies

Firstly, we intended to evaluate bedaquiline efficacy in various clinical NTM species across various MIC methods and identify bedaquiline MIC cut-off values for at least 10 clinically relevant species. However, the high heterogeneity between studies, the lack of a standardized platform for bedaquiline MIC assays in NTM species, the limited MIC₉₉ value data for individual isolates, and insufficient MIC data from various NTM species across various MIC platforms prevented us from achieving our goal.

Secondly, despite the availability of bedaquiline MIC data of over 1600 isolates using CAMHB, a true assessment could only be obtained from a few studies. Moreover, due to data being either skewed or truncated, it was difficult to determine the ECOFF value statistically using the ECOFFinder program used by EUCAST for SGM.

Thirdly, according to the ISO standard, MIC dilutions should be based on 2-fold dilution steps from 1 mg/L, i.e. 1, 0.5, 0.25 and so on and should be reported in two digits.¹⁸ Although most of the studies used a 2-fold broth microdilution method as recommended, studies were inconsistent in the MIC concentration

range tested, with some using a non-standardized (3-fold) dilution.³⁷ From the data collected, more than 100 isolates had MICs either lower than 0.008 or higher than 16 mg/L, suggesting that the range for bedaquiline MIC tests for NTM species requires further optimization. In addition, there were discrepancies in reporting certain dilutions, such as 0.12 or 0.13 for the concentration 0.125, or 0.0156 rather than 0.016.

Fourthly, most of the *in vitro* studies' methodology was not explicitly stated, and some did not report the range of MIC utilized during DST, resulting in an unclear risk of bias in the reference test in the QUADAS tool. This bias affected the review certainty and grading.

Finally, we did not assess the pharmacokinetic/pharmacodynamic (PK/PD) data in relation to bedaquiline. In fact, in absence of a uniform and standardized method for MIC determination that should be used for PK/PD, it would be difficult to obtain sufficient and reliable data for the analysis.

Therefore, we highly recommend that studies should adhere to EUCAST and CLSI guidelines when performing MIC susceptibility testing for NTM species with regard to various methodological aspects, including quality assurance measures, MIC range and dilutions reported. Additionally, studies should report the methodology section clearly to enable establishment of a standardized method specific for bedaquiline MIC with a defined MIC range and QC measures for NTM species, which are still required.

Association between genotypic and phenotypic bedaquiline resistance in NTM species

In all studies, the phenotypic assessment was applied consistently. However, around 54% (13/24) of the studies had a high risk of bias in the index domain and its applicability, due to the lack of conducting the index test (genotypic assessment). Studies that performed phenotypic and genotypic assays had unclear blinding in the genotype assessment, which could affect transparency of the data. Furthermore, not all studies investigated all genes involved in bedaquiline resistance. Variations in MIC platforms and media, the lack of a cut-off value in each MIC platform/media and the small number of isolates being sequenced are all limitations that made it difficult to determine the association between the genotypic variants and bedaquiline phenotypic resistance in various NTM species, as well as their impact on clinical outcome.

Animal model studies

The risk of bias analysis conducted as part of this systematic review indicated that many of the essential methodological details of animal model studies included in our analysis were poorly described. This resulted in an 'unclear' risk of bias for most (if not all) studies and 'high risk' of incomplete outcomes for some studies. Areas of concern noted in this review include the conflation of observation and experimental units, the representation of single datasets for repeated experiments, and the treatment of non-independent samples as independent. It is critical to highlight the importance of consistent reporting of all details of the experimental design in addition to all treatment outcomes for future animal model studies, as recommended previously in the ARRIVE guidelines.¹⁰⁵ Additionally, animal model researchers should strive for standardized preclinical models to permit comparisons. However, considering that studies that do not describe

all details may not always be methodologically faulty,⁸⁶ and because we wanted to provide a comprehensive view of the intervention effect in animal models, we still included the poorly described studies in our meta-analysis.

Due to high costs, preclinical animal model studies are often underpowered, resulting in compromised internal validity and translational value.¹⁰⁶ The limited number of the included studies and the high variations between them did not allow us to perform meta-analysis of the overall impact of the intervention. Furthermore, the lack of reporting on treatment follow-ups in most of the studies influenced the strength of this review. All the abovementioned drawbacks impact the validity of the conclusions reached.

The strength of this part of the review arises from being the first exhaustive systematic review, with a meta-analysis, on the effect of bedaquiline on decreasing bacterial burden in animal models. The extensive search and acquisition/extraction of data, the use of animal model data, highlighting the methodological limitations of the animal model studies, the use of random-effects models to account for study heterogeneity and the use of the SMD analysis to account for the variation in bacterial species and duration of treatment in meta-analysis provides context for the interpretation of outcomes of this review.

Human studies

The main limitation of the human studies included in this review is the lack of randomized clinical trials (RCTs) and controlled clinical trials (CCTs), which did not allow us to perform meta-analysis. Thus, the findings of this review cannot be regarded as conclusive evidence regarding the efficacy of bedaquiline treatments and should be interpreted with caution.

Secondly, as the included studies were case series and case reports, the variabilities between studies were very large and there was no uniformity in the methodology, duration of trial, time to culture conversion, duration of follow-up or even the therapeutic regimens used. Thus, because of various variables and discrepancies, comparing the outcomes of the studies was challenging.

Thirdly, due to the incomplete clinical data,⁶² overlapping data between studies, and lack of microbiological and *in vitro* susceptibility data in most of the studies, we could not evaluate the exact time to culture conversion or identify the correlation between the *in vitro* and the *in vivo* treatment outcome in human studies. MIC values for the isolates provide an initial assessment of antibiotic efficacy; however, in the absence of the *in vitro* assessment, translation of the MIC to *in vivo* efficacy remains unclear.

Finally, our review focused on the treatment efficacy only and we did not evaluate the safety of using bedaquiline. Nevertheless, in order to assess the drug safety, we recommend that drug safety and QTc prolongation should be evaluated and reported in more detail, even in case series and case reports.

However, despite the abovementioned limitations, this review is the first study summarizing the evidence of the efficacy of bedaquiline regimens in treating NTM-related diseases caused by the various NTM species in humans. The strengths of this part of the review lie in the extensive search and acquisition of data, and the use of individual patient data stratified by the type of

disease and NTM species. The variation among studies may indicate that the conclusions in this review are more broadly applicable.

Conclusions

In this review we compile the available data from more than 40 studies on the effect of bedaquiline on various NTM species *in vitro* and *in vivo* (animal models and human studies). We present comprehensive collective data on the MIC distribution and propose the cut-off/ECOFF value of the most clinically relevant NTM species using the CAMHB platform. Overall, bedaquiline demonstrated high antibacterial activity against almost all of the tested clinical isolates of NTM species (RGM and SGM) and was shown to have strong action against NTM species *in vitro* as well as *in vivo*. However, bedaquiline's effectiveness may vary depending on the NTM species, patient disease severity, patient immunity, drug regimen and duration of treatment.

Future research

With the increasing incidence of NTM infection and the emergence of MDR, bedaquiline could be a promising therapeutic drug alone or as part of combination regimens to treat NTM infections, particularly in NTM-related extrapulmonary infection. However, the paucity of data prevented strong conclusions regarding the ECOFF for many NTM species. This highlights the need for future studies to be standardized in terms of various factors including, but not limited to, culturing media and reporting of outcomes, to allow for meta-analysis of data. Furthermore, additional studies are critically needed to evaluate bedaquiline *in vitro* susceptibility as well as resistance in human studies, to assist in establishing the critical concentration for bedaquiline use against diverse NTM species. WHO guidelines for *M. tuberculosis* should be strictly followed in NTM infection to prevent bedaquiline-resistant strains from emerging and spreading.

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Transparency declarations

None to declare.

Data availability

Authors are agreed to share the data of this manuscript. Data will be available upon request. To request the data, contact the corresponding author of the article. Data regarding ECOFF values will be submitted to the EUCAST committee upon publication.

Supplementary data

Figures S1 to S9 and Tables S1 to S4 are available as [Supplementary data](#) at JAC Online.

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