Antimycobacterial Strategies to Evade Antimicrobial Resistance in the Nontuberculous Mycobacteria

Beverley Cherie Millar\textsuperscript{1,2,3}, John Edmund Moore\textsuperscript{1,2,3}

\textsuperscript{1}Department of Bacteriology, Northern Ireland Public Health Laboratory, Belfast City Hospital, \textsuperscript{2}Centre for Experimental Medicine, Queen’s University, \textsuperscript{3}School of Biomedical Sciences, Ulster University, Northern Ireland, UK

Abstract

The nontuberculous mycobacteria (NTM) have recently emerged as important bacterial pathogens of both animals and humans. Of particular concern is the high level of antimicrobial resistance (AMR) displayed by these organisms, which complicates treatment and potential successful outcomes. This review, therefore, wishes to examine novel compounds and approaches to combating AMR in the NTMs, specifically examining antimycobacterial (NTM) compounds from plants and venoms, as well as examining synergistic and combination effects with other antimicrobials. Novel and modified drugs including new inhaled drugs are examined, as well as the repurposing of existing drugs for antimycobacterial activity. Many of these novel interventions are at various stages of development, from initial concept through to licensed intervention. The challenge remains to translate these interventions from \textit{in vitro} laboratory models to effective \textit{in vivo} interactions. When these are realized, then we will have the opportunity of overcoming NTM AMR, to the benefit of medicine, society, and humanity.

Keywords: Antibiotic resistance, antimicrobial resistance, cystic fibrosis, nitric oxide, non-tuberculous mycobacteria, nontuberculous mycobacteria

Introduction

Recently, the nontuberculous mycobacteria (NTMs) have emerged as important human and veterinary pathogens, particularly in respiratory disease. These organisms, sometimes referred to as the atypical mycobacteria or “Mycobacteria Other Than Tuberculosis”, are usually found in water, soil, and other environmental sources and are those mycobacteria which do neither belong to the \textit{Mycobacterium tuberculosis} complex nor are \textit{Mycobacterium leprae}. At present, there are 198 species (including synonyms) of \textit{Mycobacterium}, with standing in nomenclature (http://www.bacterio.net/mycobacterium.html), of which the majority belong to the NTMs. Several key seminal review publications describing NTMs have comprehensively reviewed various aspects of NTM disease pathophysiology, epidemiology, diagnosis, treatment, and clinical management and we guide readers to these sources of information.\textsuperscript{[1-3]}

To date, none of these recent review publications have addressed novel strategies to overcome antimicrobial resistance (AMR) among the NTM organisms, and hence, it was the aim of this review to examine recent developments in overcoming AMR in NTM organisms.

Antimicrobial Resistance

One aspect to emerge from these publications is that of AMR with the NTM organisms. AMR has now emerged as a global public health crisis, with a variety of organisms, namely the \textit{Enterobacteriaceae},\textsuperscript{[4]} \textit{Pseudomonas aeruginosa}\textsuperscript{[5]} and the mycobacteria, particularly TB.\textsuperscript{[6,7]} AMR in bacteria may be manifested through several mechanisms, including alteration in cell wall permeability to antibiotics, enzymic degradation of antibiotics, efflux pump mechanisms, mutation in protein synthesis and the organism’s ability to uncoil its nucleic acid.
Table 1 summarizes the resistance mechanisms that NTM organisms adopt to evade attack from conventional antibiotic agents.

Given that the NTM organisms are environmental, it is not surprising that they have developed highly elaborate mechanisms of AMR. The environment, particularly the rhizosphere, is a harsh environment for NTMs to survive, due to highly complex inter-strain, inter-species, and inter-genus competition for habitat and nutrition. Therefore, it is advantageous that any organism can ideally perform two important functions in such a scenario, namely (i) evolve AMR mechanisms to evade natural antibiotics being excreted as secondary metabolites by neighboring bacteria, as well as (ii) having the ability to excrete novel natural antibiotic and/or antibiotic-like compounds, to suppress the growth and proliferation of neighbor bacterial organisms. While the NTMs are highly evolved in relation to developing AMR mechanisms, their ability to produce novel antimicrobial compounds is limited whereas paradoxically, another soil organisms, *Streptomyces* spp., are highly developed in producing novel antimicrobial compounds, but lack sophisticated AMR mechanisms, to evade natural antimicrobials from their neighbors in the soil.

**Mechanisms To Combat Antimicrobial Resistance in Nontuberculous Mycobacteria**

The aim of this review is to highlight recent developments which have appeared in the scientific literature within the last 2 years (2017–2018), examining a variety of approaches targeting NTMs.\[7-10\]

Medicinal plants are a source of bioactive compounds that can be effective treatments of various diseases globally. Many countries, particularly Mexico, India, Iran, Turkey, and Africa, have a wealth of medicinal plant species which have had a long standing in traditional medicine approaches to the treatment and management of diseases including tuberculosis.\[11\] Ethnobotanical/pharmacological studies relating of the use of such plants in conjunction with polyherbal medicines,\[12\] traditionally used for the treatment of tuberculosis are forming the foundations for

### Table 1: Action and resistance mechanisms of major classes of conventional antibiotics used to treat nontuberculous mycobacteria

| Drug          | Action                                         | Target                      | Resistance mechanism                                                                 |
|---------------|-----------------------------------------------|-----------------------------|--------------------------------------------------------------------------------------|
| Aminoglycosides | Inhibition of protein synthesis                 | 30S ribosomal unit (16S rRNA) | Steric hindrance by the addition of acyl and phosphate groups                          |
|               | Differing mode of action depending on chemical structure of drug |                            | Enzymatic drug inactivation of drug by aminoglycoside modifying enzymes AAC[2'] and Eis2 |
|               | -Inhibition of translocation of tRNA-mRNA      |                            | Altered target binding Phosphotransferase (APH[33'])                                 |
|               | -Protein mistranslation                        |                            | Low-cell permeability                                                                |
|               | -Interference with the delivery of aminoacylated tRNA to the A-site |                            | Efflux pumps (tetV , tap, P55)                                                      |
|               |                                               |                            | Mutated gene involvement 16S rRNA                                                    |
| Beta-lactams   | Inhibition of cell wall synthesis               | Penicillin-binding protein  | Enzymatic drug degradation                                                           |
|               |                                               |                            | Hydrolytic β-lactamase enzyme (Bla<sub>mp</sub>)                                     |
|               |                                               |                            | Low-membrane permeability                                                            |
| Fluoroquinolones/ | Inhibition of DNA synthesis                    | DNA gyrase                  | Polymorphisms in target gene gyr A, gyr B                                            |
| Macrolides     | Inhibition of protein synthesis by preventing the elongated polypeptide chain from vacating the peptidyltransferase center of the ribosome | Nascent polypeptide tunnel within the 50S ribosomal subunit (23S rRNA) | Efflux pumps (MAY<sub>1406</sub>, MAY<sub>3306</sub>, MAY<sub>1695</sub>) |
| Oxazolidinone  | Inhibition of protein synthesis                 | 50S ribosomal subunit (23S rRNA) | Erythromycin ribosome methylase (Erm) enzyme methylates A2058 nucleotide (23S rRNA gene) lowering macrolide binding by preventing the elongated affinity for exit tunnel |
| Rifampicin/ rifabutin | Inhibition of transcription                  | β-subunit of RNA polymerase | Acquired resistance due to mutation in the 23SrRNA gene, a base change at either position 2058 or 2059, critical rRNA residues involved in the binding of macrolides to ribosome |
|               |                                               |                            | Mutated gene involvement 23S rRNA                                                   |
| Tetracycline   | Inhibition of protein synthesis by interference with the delivery of aminoacylated tRNA to the A-site | 30S ribosomal unit          | Drug efflux                                                                          |
|               |                                               |                            | Ribosome protection                                                                  |
|               |                                               |                            | Enzymatic inactivation and decomposition                                              |
|               |                                               |                            | -Tetracycline-degrading flavin monoxygenase (MabTetX)                                 |

Information taken from References\[7-10\]
the identification and in vitro examination of antibacterial properties against NTMs [Table 2].

Animal venoms from snakes (terrestrial and sea), scorpions, spiders, honey bees, wasps, and snails, have been investigated and found to be a rich source of natural antimicrobial substances including proteins, amines, bioactive peptides, antimicrobial peptide (AMP), toxins and enzymes, showing activity, by a number of different mechanisms, against many pathogens and more recently, NTMs [Table 3].

Of particular, clinical interest has been the AMPs which are short (10–15 amino acid residues), due to the fact that they are structurally stable, do not easily induce AMR compared to conventional antimicrobials and have shown potent potential in killing bacteria, fungi, viruses, and parasites. AMPs are currently a source of therapeutic potential particularly as they are devoid of hemolytic properties, not toxic to host cells and may be readily synthesized and modified.

Owing to the intrinsic resistance of NTMs to most commonly used antibiotics, such infections are treated by a multidrug regimen as highlighted in the recent “British Thoracic Society Guidelines for the management of non-tuberculous mycobacterial pulmonary disease,” however, treatment issues are further complicated by the ability of Mycobacterium abscessus to develop macrolide resistance on exposure to sub-inhibitory concentrations of the drug or where other members of the macrolides are used, such as in the case of low-dose azithromycin in the management of patients with cystic fibrosis (CF). Currently, screening for synergistic interactions of approved drugs is an approach which has identified novel in vitro synergistic combinations, with one of the largest studies totaling 180 dual drug combinations against M. abscessus, reported recently by Aziz et al. 2018. Table 4 summarize several studies which report on potential synergistic and combination effects with other antimicrobials.

Of interest, has been a recent publication examining the interaction between spices and antibiotic resistance in M. abscessus. In this study, M. abscessus isolates (n = 9 multidrug-resistant clinical isolates from CF patients + 1 reference strain) were examined for their direct susceptibility to 27 spices, as well as the interactive effect of this spice combination to their susceptibility to amikacin and linezolid antibiotic, with standard disk diffusion assay. Five isolates of M. abscessus (5/10; 50%) failed to grow on the spice enriched medium, which included four clinical isolates and the National Culture Type Collection Reference Strain. Of the remaining five isolates which grew on the spice medium, no cultural phenotypic differences were observed, compared to unsupplemented controls. In the case of both amikacin and linezolid, the zone of inhibition increased with the inclusion of the spices. Initially, all isolates of M. abscessus were fully resistant to linezolid (mean zone of inhibition = 0 mm), and growth was to the edge of the antibiotic disk, whereas when in the presence of spices, large zones of inhibition were observed (mean zone of inhibition = 33.3 mm). With amikacin, the mean zone of inhibition increased from 23.2 mm to 32.0 mm, in the presence of spices. These data suggest that the spices were interacting synergistically with the antibiotics, thus making the antibiotic more potent against the bacteria tested. This study is significant as it demonstrates a positive interaction between spices and the conventional antimycobacterial antibiotics, amikacin, and linezolid. Given the burden of AMR to M. abscessus, particularly in a patient with chronic disease such as CF, any food-related innovation that can help maximize the potency of existing antimycobacterial antibiotics is to be encouraged and developed. The specific mechanism as to how spices increase the potency of such antibiotics with M. abscessus now needs to be elucidated, as well as novel food (spice) delivery modalities developed, including novel medicinal foodstuffs or functional foods, that can harness this beneficial effect in vivo.

Due to the urgent need to address the AMR of NTMs, it is important to discover new antimycobacterials, however to date, such drug discovery has been limited, particularly in relation to progression of such novel drugs to clinical trials [Table 5].

Of particular, interest has been the development of a novel inhaled nonantibiotic therapy, nitric oxide gas formulation, Thiolanox® (Novoteris, Garden Grove, CA, USA), for the treatment of CF and which is currently in Phase II development (NCT02498535). Interestingly, in Phase I of the clinical study, with CF patients, 160ppm NO inhaled for 30 min, three times daily for five days over two consecutive weeks, indicated that this therapy was safe and significantly reduced the number of various bacteria including M. abscessus, which consequently reduced pulmonary inflammation and increased lung function to levels not commonly observed after antibiotic therapy (Table 6). It has been suggested that NO has a multiplicity of targets that are non-organism specific, attributed to its oxidative and nitrosylating effects. NO eradicates microbes by nitrosylating their heme-or thiol-containing essential metabolic proteins which interfere with RNA replication and DNA repair mechanisms, which in turn damages cellular structure and function and modulates the host immune response. Subsequently, an interventional clinical trial is currently in progress to investigate if NO therapy can reduce the NTM bacterial load in the lungs of adults and adolescents with NTM infection (NCT03331445), the preliminary results of which are summarized in Table 6. Furthermore, the antibacterial efficacy of a biopolymer, NO-donor BIOC51 (Vast Therapeutics, Chapel Hill, NC, USA) has been demonstrated against NTMs in a mouse in vitro and in vivo model.

It is important to consider the fact that NTM can exist both extracellularly in biofilms and intracellularly within macrophages and other host cells, where they can replicate intracellularly. This promotes a challenge when delivering antimicrobial therapy at concentrations which would be effective against NTMs persisting either within biofilms or intracellularly within host cells. Of significant interest has been the development of a liposomal amikacin formulation.
**Table 2: Pharmacognosy relating to the antimycobacterial activity of plant extracts**

| Natural product                                                                 | Study findings                                                                                                                                                                                                 | Species used              |
|--------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------|
| **EOs from medicinal plants- Lamiaceae family (Satureja rechingeri, Satureja khuzestanica, Zataria multiflora)**[13] | M. tuberculosis was completely inhibited by Z. multiflora at 78 μg/ml. Hexane, CHCl₃, EtOAc, and ethanol extracts had antibacterial activity against M. tuberculosis H37Rv. Ethanol extract subjected to fractionation by selective extractions, purified compound of phenolic nature obtained (MIC: 3.125 μg/ml against M. tuberculosis). | M. tuberculosis, M. kansasii, M. fortuitum, M. smegmatis, M. aurum, M. tuberculosis |
| **Bidens odorata** Cavanilles, a medicinal and edible plant[14]                  | Hexane and dichloromethane extracts were effective against M. smegmatis. Aqueous crude extract did not display antimycobacterial activity, but following fractionation, isolation of 3,5- hydroxybenzoic acid, showed biological activity against M. tuberculosis. | M. smegmatis               |
| **Medicinal herbal plants (Senegal) (Combretum aculeatum and Guiera senegalensis)**[15] | Aqueous extracts demonstrated significant antimycobacterial activities. IC₅₀ G. senegalensis (0.098±0.009 mg/ml) and C. aculeatum (0.074±0.006 mg/ml), compared to Rifampin (0.007±0.0004 mg/ml). | M. marinum                |
| **Medicinal plants (Datura stramonium, Boswellia serrata, Lavandula stoechas, Rosmarinus officinalis, Thymus vulgaris)**[16] | Crude ethanol extract showed antimycobacterial activity (MIC: 125-500 μg/ml). Hexane and butanol sub-fractions of A. dimidiata exhibited potent anti-mycobacteria activity. | M. bovis                  |
| **Combined crude extracts of medicinal plants (Limpopo Province, South Africa) Combretum hereroense, Citrus lemon Apodytes dimidiata**[17] | MICs; crude extracts (0.1-3 mg/ml), average synergistic effect of the plants (0.04 mg/ml-1.25 mg/ml). Hexane and butanol sub-fractions of A. dimidiata exhibited potent anti-mycobacteria activity. | M. smegmatis               |
| **Alkaloid extracts from Combretum zeyheri**[18]                               | MIC value of 125 μg/ml. Other Combretum species examined were not as potent. | M. smegmatis               |
| **Curcumin, a phenolic compound extracted from Curcuma longa**[19]              | MIC=128 mg/L. Synergic effect of curcumin with AMK, clarithromycin, ciprofloxacin and LZD (strain initially showed resistance/intermediate susceptibility). Curcumin (1/8×MIC) significantly reduced motility and 4 × MIC, complete inhibition of 4- and 8-day mature biofilms. Synergistic combinations of curcumin and AMK induced a reduction in microbial aggregates and substantial loss in cell viability. | M. abscessus               |
| **Nanoemulsions of Cymbopogon flexuosus**[20]                                  | Nanoemulsion exhibited significant antimicrobial activity with MICs lower than those of the free EO, against strains in the planktonic state. | M. fortuitum, M. massiliense, M. abscessus, M. smegmatis |
| **Phytochemicals from Parinari curatellifolia leaf extracts**[21]              | MICs; 6.2 μg/ml for the acetone extract, 12.5 μg/ml for both the ethanol and the total extract and 50 μg/ml for both the methanol and ethyl acetate extracts. | M. smegmatis               |
| **Stem bark of Tetracera potatoria Afzel. Exg. Don (Dilleniaceae) medicinal plant used traditionally in Africa**[22] | Tetraceranoate exhibited the best activity against M. smegmatis with a minimum MIC of 7.8 μg/mL. β-stigmasterol, betulinic acid and betulin showed appreciable anti-mycobacterial activity against both strains (MIC 15 μg/mL). | M. smegmatis, M. aurum     |
| **Persimmon (Ebenaceae Diospyros kaki Thunb.) derived tannin**[23]             | Soluble tannin hydrolysate exhibited high bacteriostatic activity against MAC in vitro. MAC infected mice fed a soluble tannin-containing diet showed significantly higher anti-bacterial activity against MAC than control fed animals. Levels of pro-inflammatory cytokines and iNOS were significantly reduced by treatment with soluble tannin hydrolysate. | MAC                       |
| **Rhynchosia precatoria (Humb. and Bonpl. ex Willd.) (Fabaceae) DC. Medicinal plant**[24] | New isoflavonones identified. Antimycobacterial and synergistic antimycobacterial activity noted. | M. tuberculosis, M. smegmatis |

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to address these issues and in particular liposomal inhalation suspension which can be delivered by to target NTM lung diseases. Such delivery mechanisms ensure the delivery of high concentrations of antibiotic directly to the lung along with low systemic concentration in an attempt to prevent cytotoxicity.\textsuperscript{[83]} In vitro and in vivo animal models have shown the ability which this liposomal inhalation suspension has in penetrating NTM biofilms, as well as enhancing amikacin uptake into macrophages.\textsuperscript{[81]} Of major clinical interest has been a multi-centered clinical trial (NCT02344004) which concluded that a single daily nebulization of amikacin liposome inhalation suspension (590 mg), when added to standard guideline-based therapy (GBT) in patients with refractory Mycobacterium avium complex (MAC) lung disease, achieved significantly greater culture conversion by month 6 (defined as three MAC-negative sputum cultures) than GBT alone, along with comparable rates of serious adverse events.\textsuperscript{[82]} These findings further highlight the importance of novel inhaled therapeutic approaches for the treatment of MAC lung disease.

Another approach to tackle the challenge posed by NTM AMR has been the repurposing of existing drugs, namely those that had been approved previously for the treatment of tuberculosis, such as bedaquiline, clofazimine, rifabutin and skin infections, such as tedizolid [Table 7]. Of concern, however, is that fact the resistance mechanisms associated with the MmpL family of proteins have been identified in M. abscessus, in the case of clofazimine and bedaquiline, where clofazimine-resistant strains demonstrated cross resistance to bedaquiline.\textsuperscript{[90,91,97]}

In addition, in vitro, the antimicrobial effect on NTMs as a result of polypharmacy for the treatment of noninfective conditions has been noted and opens up another avenue to peruse [Table 7].

Table 8 highlights a number of other approaches which have been investigated in the search for antimycobacterial drugs for NTMs including the examination of existing screening libraries and the examination of bacterial virulence and pathogenic mechanisms with the potential to develop antivirulence therapies and bacteriophage therapy.

Bacteriophage therapy is an interesting approach to consider in the fight against AMR, however to date research in the potential use of such therapy in the case of NTM has been limited with recent focus associated with M. tuberculosis.\textsuperscript{[105]} Indeed, phage therapy although extensively used in Eastern Europe in relation

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**Table 2: Contd...**

| Natural product | Study findings | Species used |
|----------------|---------------|--------------|
| **Berries** | | |
| Juniper (EO)\textsuperscript{[25]} | MEC 1.6 mg/mL Significant reduction in cell viability of *M. intracellulare* and *M. gordonae* at MEC and of *M. avium* at 2 × MEC Microscopic analysis confirmed inhibitory effect, revealing significant morphological changes in the cell membrane and cytoplasm of all bacteria Mode of action on the cell membrane confirmed by marked leakage of intracellular material | *M. avium* spp. *avium* |

| **Fungi/mushroom** |  | |
| Lentinus citrinus Walley and Rammeloo DPUA 1535 Neolentinus lepideus (Fr.) Redhead and Gims DPUA 1536 Pleurotus ostreatus (Jacq.; Fr.) Kumm. (DPUA 1533) P. ostreatus (Jacq.; Fr.) Kumm. cv. Florida (DPUA 1534)\textsuperscript{[21]} | Organic mycelial extracts of *Lentinus* and *Pleurotus* species exhibited potential antibacterial and antifungal activity | *M. smegmatis* |

| **Marine sources** |  | |
| Bioactive Pyridone Alkaloids from Deep-Sea-Derived Fungus Arthrinium spp. UJNMF000\textsuperscript{[27]} | Eight new 4-hydroxy-2-pyridone alkaloids arthpyrones and two known analogs (apiosporamide and arthpyrone B) were studied | *M. smegmatis* |
| Dimeric 3-alkyl pyridinium alkaloids (halicycloclamelines) and analogs (cyclolesteltettamines) from Indonesian marine sponge Halicloina spp.\textsuperscript{[29]} | Anti-mycobacterial activity noted | *M. smegmatis* |
| Bisfunctionalized sphingolipid (leucettamol A) from Indonesian marine sponge Agelas spp.\textsuperscript{[29]} | Leucettamol A- moderate anti-mycobacterial activity (50 µg/disk, 20 µg/disk and 10 µg/disk inhibition zones: 12, 9 and 7 mm, respectively) Free amino groups were important for anti-mycobacterial activity | *M. smegmatis* |

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**M. smegmatis**: Mycobacterium smegmatis, *M. kansasii*: Mycobacterium kansasii, *M. gordonae*: Mycobacterium gordonae, *M. tuberculosis*: Mycobacterium tuberculosis, *M. fortuitum*: Mycobacterium fortuitum, *M. intracellulare*: Mycobacterium intracellulare, *M. aurum*: Mycobacterium aurum, *M. abscessus*: Mycobacterium abscessus, *M. massilense*: Mycobacterium massilense, *M. bovis*: Mycobacterium bovis, MICs: Minimum inhibitory concentrations, MAC: Mycobacterium avium complex, MEC: Minimal effective concentration, EO: Essential oil, FICI: Fractional inhibitory concentration index, *A. dimidiata*: Apodytes dimidiata, *Mycobacterium avium*, *M. marinum*: Mycobacterium marinum, AMK: Amikacin, LZD: Linezolid, iNOS: Inducible nitric oxide synthase
Table 3: Venom-derived antimicrobial peptides

| Antimicrobial Peptide                                                                 | Study findings                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Species used   |
|-------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------|
| ToAP2 (derived from scorpion *Tityus obscurus*)[^31]                                  | ToAP2 inhibited the growth of *M. massiliense* at a MBC of 200 μM. MBC concentration used to treat infected macrophages was able to inhibit 50% of the bacterial growth of all strains. ToAP2 treatment of infected mice with bacilli reduced the bacterial load in the liver, lung, and spleen, similar to clarithromycin levels (90%). The *in vitro* antimicrobial activity of ToAP2 is improved *in vivo* due to chemotactic activity. | *M. massiliense* |
| NDBP-5.5 (derived from scorpion *Hadrurus gertschi*)[^32]                            | MBC 200 μM which did not induce hemolysis of red blood cells. NDBP-5.5 had a low toxicity and therefore good clinical potential (*therapeutic index 3.05*). Treatment of infected macrophages with NDBP-5.5 or clarithromycin presented similar results, reducing the bacterial load in *M. abscessus subsp. massiliense*-infected animals showed a decrease in the bacterial load of up to 70% when treated with NDBP-5.5. | *M. abscessus subsp. massiliense* |
| AMPs developed from CTX-1 of the Chinese cobra (*Naja atra*)[^33]                    | Potent antimicrobial activity was demonstrated                                                                                                                                                                                                                                                                                                                                                                                                                               | *M. smegmatis* |
| Polybia-MPII, a mastoparan peptide from the female Neotropical social wasp *Pseudopolybia vespiceps testacea* (Vespidae, Hymenoptera)[^34] | The mastoparan reduced *in vitro* and *ex vivo* (macrophage murine model) mycobacterial growth by 80% at 12.5 μM in infected peritoneal macrophages but did not affect the shape of bacterial cells at the dose tested (6.25 μM). Polybia-MPII as a therapeutic alternative demonstrated remarkable potential to inhibit mycobacteria and to penetrate the cell wall to kill bacteria from inside the cytoplasm. | *M. abscessus subsp. massiliense* |
| Polydim-I, from the female Neotropical wasp *Polybia dimorpha*[^35]                  | Polydim-I iv. treatment of with *M. abscessus subsp. massiliense* induced 0.8-1 log reduction of the bacterial load in the lungs, spleen and liver. Noncytoxotoxicity toward mammalian cells. Polydim-I acted on the *M. abscessus subsp. massiliense* cell wall and reduced 40%-90% of the bacterial load both *in vitro* and *in vivo*. | *M. abscessus subsp. massiliense* |

*M. massiliense*: *Mycobacterium massiliense*, MBC: Minimal bactericidal concentration, AMP: Antimicrobial peptide, NDBP: Non-disulphide bridge peptide, CTX-1: Cardiotoxin 1, IV: Intravenous, *M. abscessus*: *Mycobacterium abscessus*, *M. smegmatis*: *Mycobacterium smegmatis*, *M. fortuitum*: *Mycobacterium fortuitum*

Table 4: Potential synergistic and combination effects with other antimicrobials

| Combinations                                                                 | Study findings                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Species used   |
|------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------|
| Synergistic antibiotic combinations capable of overcoming drug resistance *in vitro*[^36] | *In vitro* activity of several antibiotics against a selection of drug-resistant NTM clinical isolates from CF patients and paired combinations of antibiotics against a subset of *M. abscessus* strains. Clofazimine and clarithromycin exhibited 100% synergy for all combinations tested, as did AMK, with the exception of one isolate. | *M. abscessus* |
| *Synergistic effect of LZD with AMK, MOX, CFX and TGC*[^38] | LZD and AMK most potent synergistic activity. Frequent synergism in LZD-AMK and LZD-TGC. LZD rarely exhibited *in vitro* synergy with MOX and CFX when tested against MABC. LZD-CFX and LZD-MOX combinations antagonistic for half of the isolates. | *M. abscessus* |
| Clarithromycin-vancomycin[^40]                                                | Strong synergy was found with a FICI score of ≤0.5 and a 4-to-10-fold decrease in MIC.                                                                                                                                                                                                                                                                                                                                                                                      | MABC (subspecies) |
| Thioridazine/MOX -based combination regimen[^41]                             | Rapid microbial kill could be achieved within 7 days                                                                                                                                                                                                                                                                                                                                                                                                                        | *M. avium-intracellulare complex* |
| Ceftazidime/avibactam, rifabutin, TZD and MOX[^42]                           | Kill rates better than standard therapy                                                                                                                                                                                                                                                                                                                                                                                                                                   | *M. avium subsp. hominisuis* |
| Ceftazidime/avibactam[^41]                                                   | Ceftazidime in combination with the non-β-lactam β-lactamase inhibitor avibactam kills MAC. Microbial kill was better than that of standard therapy drugs at currently recommended doses                                                                                                                                                                                                                                                    | MAC |

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### Table 4: Contd...

| Combinations                               | Study findings                                                                 | Species used       |
|--------------------------------------------|--------------------------------------------------------------------------------|--------------------|
| Avibactam and various carbapenems[^44]     | The addition of avibactam to various carbapenem antibiotics effectively reduced the MICs of carbapenem-resistant *M. abscessus* isolates to within therapeutically achievable levels *in vitro* | *M. abscessus*     |
| Clarithromycin, rifampin, rifabutin, and ethambutol in combination with ATP[^45]   | *In vitro* anti-*Mycobacterium* complex activity of combination therapies was expressed in a strain-dependent manner *In vitro* regrowth of drug-treated bacteria was delayed by combined use of ATP | MAC                |
| Synergistic effect of Clarithromycin with LZD, MOX , AMK, and tigecycline[^46] | *In vitro*, synergistic activity was noted with clarithromycin and various other drugs | *M. abscessus*     |
| Rifampicin with hydroperoxides[^47]       | Increased membrane permeability owing to the presence of the oxidant, led to higher uptake of the drug Additive effect was noted | *M. bovis* *M. massiliense* *M. tuberculosis* |
| Interaction of South Asian spices with conventional antibiotics[^48] | Synergistic antimicrobial activity noted *in vitro* between spice extracts and AMK and LZD | *M. abscessus*     |
| Teicoplanin - Tigecycline combination[^49] | *In vitro* checkerboard titration assay Synergistic activity observed | *M. abscessus Bamboo* *M. abscessus subsp. abscessus, massiliense, bolletii* |

NTM: Non-tuberculous mycobacteria, LZD: Linezolid, AMK: Amikacin, MOX: Moxifloxacin, CFX: Cefoxitin, TGC: Tigecycline, ATP: Adenosine 5′-triphosphate, FICI: Fractional inhibitory concentration index, *M. abscessus*: *Mycobacterium abscessus*, *M. smegmatis*: *Mycobacterium smegmatis*, *M. tuberculosis*: *Mycobacterium tuberculosis*, *M. chelonae*: *Mycobacterium chelonae*, *M. bovis*: *Mycobacterium bovis*, *M. massiliense*: *Mycobacterium massiliense*, MAC: *Mycobacterium avium* complex, MICS: Minimum inhibitory concentration, CF: Cystic fibrosis, TZD: TzDizolid

### Table 5: The antimycobacterial activity of novel drugs/compounds and modified drugs

| Novel drugs/compounds | Study findings                                                                 | Species used       |
|-----------------------|--------------------------------------------------------------------------------|--------------------|
| Nitrogen heterocycles derivatives: 7-(pyridine-4-yl)-indolizine derivatives[^49] | Potent derivatives against both replicating and non-replicating *M. tuberculosis* Bactericidal mechanism of action Active against drug-resistant MTB strains Moderate to good activity against NTMs Good intracellular activity Moderate to high cytotoxicity | *M. tuberculosis* *M. abscessus* *M. avium* |
| Potential new 3-phenylquinolone efflux pump inhibitors based on natural isoflavone biochanin A[^50] | 3-phenylquinolones inhibited *M. avium* efflux pumps A protonable N-1 aminoalkyl chain was very important, with a tertiary cyclic amine to give the best inhibitory efflux pump activity Synergistic activity was noted with reference to macrolides and fluoroquinolones | *M. smegmatis* *M. avium* |
| Diphenyleneiodonium chloride (DPIC), an NADPH/NADH oxidase inhibitor[^51] | MIC of 0.125-0.25 mg/L Concentration-dependent bactericidal activity against *M. fortuitum* was comparable to AMK but outcompeted meropenem No regrowth after treatment with DPIC (10× MIC) Greater ability to eradicate intracellular mycobacteria as compared with AMK DPIC synergistic effect with all the tested drugs (AMK, ceftriaxone, ceftazidime, and meropenem) against *M. fortuitum* | *M. fortuitum, M. avium, M. chelonae, M. abscessus* |
| Indole-2-carboxamides[^52] | Act by inhibition of MmpL3, an essential transporter required for the translocation of mycolic acids to the mycobacterial cell envelope Pan anti-mycobacterial activity Selective for mycobacteria Minimal in vitro cytotoxicity | *M. abscessus* *M. massiliense*, *M. bolletii, M. chelonae*, *M. tuberculosis, M. avium*, *M. xenopi, M. smegmatis* |
| Synthetic antimicrobial polymers derived from a biocompatible polyamide backbone with quaternary amine pendants of varied hydrophobicity[^53] | Bactericidal activity toward drug-sensitive and drug-resistant mycobacteria High specificity targeting both intracellular and biofilm forms of mycobacteria | *M. smegmatis M. bovis* (BCG) *M. tuberculosis* Drug resistant *MTb* clinical isolates |

Contd...
### Table 5: Contd...

| Novel drugs/compounds | Study findings                                                                                                                                                                                                                                                                                                                                                   | Species used                                                                 |
|-----------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------|
| TZD - a new oxazolidinone (Target 50S ribosome) | **MIC**< sub>< sub> and **MIC**< sub>< super> of TZD (2 and 8 μg/mL, respectively); 2-16-fold lower than LZD  
No difference between the MAC subspecies  
Time-kill assays did not show any bactericidal activity at 4- and 8-fold the MIC  
Combination of TZD with clarithromycin was synergistic (1 out of 6 isolates), while indifferent interactions for TZD combined with tigecycline, ciprofloxacin, and AMK | *M. abscessus* subsp. (*abscessus, massiliense, bolletii*)                     |
| TZD, a next-generation oxazolidinone | **In vitro** kill time analysis using 130 isolates                                                                                                                                                                                                                                                  | *M. abscessus*                                                             |
|                        | TZD exhibited a bacteriostatic effect that was more pronounced in *M. bolletii* and *M. massiliense* than *M. abscessus*                                                                                                                                                                          | *M. massiliense*                                                          |
|                        | TZD exhibited little concentration-dependent killing and no significant bactericidal activity                                                                                                                                                                                                  | *M. bolletii*                                                              |
| TZD, 50S ribosome target | **Susceptibility breakpoint for TZD (200 mg/day) was 1 mg/L, above which patients were likely to fail therapy; 2 mg/L was a susceptible-dose-dependent breakpoint**                                                                                                                                                                                                 | *M. avium subsp.* *hominissuis*                                              |
| Inhibitors Against Mycobacterial Protein Kinase G (PknG) | PknG is a eukaryotic-like serine/threonine kinase that is expressed by *Mycobacterium tuberculosis* and promotes survival of mycobacteria in host macrophages by suppressing phagosome-lysosome fusion  
PknG inhibitors identified | *M. bovis*                                                                 |
| Novel Oxazolidinone with a cyclic amidrazide (LCB01-0371, LegoChem BioSciences, Inc. (Daejeon, Republic of Korea)) | LCB01-0371 was effective against several *M. abscessus* strains  
in **in vitro** and in a macrophage model of infection  
LCB01-0371 inhibited the growth of AMK-, CFX - and clarithromycin-resistant strains | *M. abscessus*                                                             |
| LZD, 50S ribosome target | The clinical dose of 600 mg/day achieved or exceeded the bacteriostasis exposure in 98.73% of patients  
The proportion of 10000 patients Monte Carlo simulations treated with the standard 1200 mg/day who achieved the exposure for 1.0 log10 cfu/mL kill was 70.64%, and 90% for 1800 mg/day  
**MIC for the laboratory strain was 4.0 mg/L**  
The proposed MIC breakpoint for LZD is 16 mg/L, with which 49%-80% of clinical isolates would be considered resistant | *M. avium Chester*                                                         |
| Sulfamethoxazole ureas and oxalamide | **MIC values starting from 2 μM**  
Several derivatives exhibited an antimycobacterial activity comparable or superior to sulfamethoxazole and isoniazid  
Methyl, cyclopropyl and 4-(N-(5-methylisoxazole-3-yl) sulfamoyl) phenyl were favored as the 3-ureido substituents of sulfamethoxazole-based urea | *M. tuberculosis*                                                          |
| Rhodamine-3-acetic acid derivatives | All of the derivatives were active against mycobacteria even isoniazid-resistant atypical mycobacteria | *M. avium*                                                                |
| Piperidinol derivatives | The piperidinyld and the bis-Mannich base analog were found to be selective for mycobacteria and rapidly kill this organism with a cytotoxicity selectivity index for mycobacteria of >30-fold | *M. smegmatis*                                                            |
| Thiosemicarbazide Derivatives | Most of the compounds showed good activity against the test organism | *M. bovis*                                                                |
| Fluorene Bisamide Derivatives | Most active compound (MIC of 1.95 μg/mL) against *M. bovis* | *M. tuberculosis*                                                          |
| Sesamol (Ses) a natural phenolic compound | MIC 6mM  
Results indicated that Ses is involved in the disruption of the membrane integrity of Mycobacteria and also induces reactive oxygen species | *M. smegmatis*                                                            |

*Contd...*
to other pathogenic organisms, is limited elsewhere globally. In
general clinical trials, in relation to phage therapy, have been
sparse, primarily due to safety concerns relating to the sterility
and purity of phages and the potential onset of toxic shock due
to the bactericidal effect of phages. Furthermore, regulatory
guidelines relating to their therapeutic use require clarification.\textsuperscript{[106]}

### Table 5: Contd...

| Novel drugs/compounds | Study findings | Species used |
|-----------------------|--------------|--------------|
| NO-donor modified from a natural biopolymer that releases NO spontaneously in solution (BIOC51; Vast Therapeutics, USA)\textsuperscript{[66]} | In vitro MIC and MBC assays, In vivo mouse model. BIOC51 killed NTMs investigated. In vitro BIOC51 significantly reduced \textit{M. abscessus} levels (by 2.4 logs). BIOC5 was fast acting with no adverse effects | \textit{M. abscessus}, \textit{M. avium}, \textit{M. intracellular} |
| Diaryltriazene derivatives\textsuperscript{[67]} | FOR \textit{M. avium} and \textit{M. abscessus} under iron-free conditions. Ga\textsubscript{3}O\textsubscript{4} (MICs: 8 μg/mL (31 μM) and 32 μg/mL (125 μM), respectively) Ga\textsubscript{3}O\textsubscript{4} inhibited the growth (1–8μg/mL and 4–8μg/mL, except the UNMC1374 strain. Traphenylporphyrin alone did not inhibit the growth of NTMs in iron-free media. Ga\textsubscript{3}O\textsubscript{4} was more effective than GaNP in inhibiting the growth. GaNP inhibited the growth of either NTM under iron-rich conditions. GaNP had better and more prolonged (up to 15 days) activity against NTMs growing within the THP-1 macrophage cell line. Ga\textsubscript{3}O\textsubscript{4} and GaNP exhibited inhibitory activities via interruption of iron acquisition during intracellular and extracellular infection. | \textit{M. smegmatis} |
| Free and Nanoparticle Formulations of Gallium (III) meso-Tetraphenylporphyrin (Ga(NO\textsubscript{3})\textsubscript{3}), GaCl\textsubscript{3}, gallium meso-tetraphenyl porphyrin (GaTP), and gallium nanoparticles (GaNP)\textsuperscript{[68]} | MIC 0.03 μg/mL for \textit{M. avium} and \textit{M. abscessus} under iron-free conditions. Ga\textsubscript{3}O\textsubscript{4} (MICs: 8 μg/mL (31 μM) and 32 μg/mL (125 μM), respectively) Ga\textsubscript{3}O\textsubscript{4} inhibited the growth (1–8μg/mL and 4–8μg/mL, except the UNMC1374 strain. Traphenylporphyrin alone did not inhibit the growth of NTMs in iron-free media. Ga\textsubscript{3}O\textsubscript{4} was more effective than GaNP in inhibiting the growth. GaNP inhibited the growth of either NTM under iron-rich conditions. GaNP had better and more prolonged (up to 15 days) activity against NTMs growing within the THP-1 macrophage cell line. Ga\textsubscript{3}O\textsubscript{4} and GaNP exhibited inhibitory activities via interruption of iron acquisition during intracellular and extracellular infection. | \textit{M. smegmatis}, \textit{M. avium}, \textit{M. abscessus} |
| Diaryltriazene derivatives\textsuperscript{[67]} | Compound, isolated from the fermentation broth of a locust-associated \textit{Streptomycyes} spp. showed weak antibacterial activity, 85.6 μg/mL | \textit{M. smegmatis} |
| Diaryltriazene derivatives\textsuperscript{[67]} | Most active carboxamides were substituted by short n-alkyl (MICs against \textit{Mtb}. of 0.5-2 μM). Cyclization did not increase activity. 2-isonicotinoylhydrazine-1-carboxamides mechanism of action described as inhibition of enoyl-ACP reductase (InhA), similar to INH, which blocks the biosynthesis of mycolic acids. N-Dodecyl-5-(pyridin-4-yl)-1,3,4-oxadiazol-2-amine was the most efficacious oxadiazole inhibiting growth of \textit{Mtb} (susceptible and drug-resistant) with MIC values of 4-8 μM. | \textit{M. tuberculosis} (sensitive, MDR and XDR), \textit{M. avium}, \textit{M. kansasii} |
| Diaryltriazene derivatives\textsuperscript{[67]} | N-acetylated fluorophenylalanine-based aromatic amides and esters\textsuperscript{[73]} | The amidic derivatives showed no significant antimycobacterial properties. The majority of the esters exhibited a mild antimycobacterial activity. | \textit{M. tuberculosis}, \textit{M. avium}, \textit{M. kansasii} |
| Diaryltriazene derivatives\textsuperscript{[67]} | Isoniazid-based hydrazones\textsuperscript{[74]} | Characterization and evaluation of antibacterial, antifungal, antimycobacterial, cytotoxic and cytostatic action. | \textit{M. tuberculosis}, \textit{M. avium}, \textit{M. kansasii} |
| Diaryltriazene derivatives\textsuperscript{[67]} | Schiff bases derived from the sulfa drug sulfadiazine and various salicylaldehydes-4-(2-hydroxybenzylidene)aminol-N-(pyrimidin-2-yl) benzene-sulfonamides\textsuperscript{[75]} | \textit{M. tuberculosis} and \textit{M. kansasii} were inhibited within the range of 8-250 μM. | \textit{M. tuberculosis}, \textit{M. avium}, \textit{M. kansasii} |

CFX: Cefoxitin, DPIC: Diphenyleneiodonium chloride, \textit{M. tuberculosis}: \textit{Mycobacterium tuberculosis}, \textit{M. chelonae}: \textit{Mycobacterium chelonae}, \textit{M. avium}: \textit{Mycobacterium avium}, \textit{M. kansasii}: \textit{Mycobacterium kansasii}, \textit{M. bovis}: \textit{Mycobacterium bovis}, TZD: Tedizolid, \textit{M. smegmatis}: \textit{Mycobacterium smegmatis}, \textit{M. intracellular}: \textit{Mycobacterium intracellular}, \textit{M. marinum}: \textit{Mycobacterium marinum}, MICs: Minimum inhibitory concentrations, MBC: Minimal bactericidal concentration, LZD: Linezolid, AMK: Amikacin
In conclusion, AMR in NTM organisms presents significant clinical treatment dilemmas and challenges, for a range of infections associated with NTMs. This review presents a synthesis of novel and innovative approaches, as described in Tables 2-8, in an attempt to circumvent such AMR problems. These approaches are at various stages of development, from initial concept through to licensed intervention. The challenge remains to translate these interventions from in vitro laboratory models to effective in vivo interactions. When these are realized, then we will have the opportunity of overcoming NTM AMR, to the benefit of medicine, society, and humanity.

### Table 6: Novel Inhaled therapies examined for treatment of nontuberculous mycobacteria

| Inhaled therapy | Study findings | Species used |
|-----------------|----------------|--------------|
| **NO**<sup>[78]</sup> | Prospective compassionate adjunctive inhaled NO therapy (160 ppm) in 2 CF patients with persistent *M. abscessus* infection Significant reduction of *M. abscessus* load (7000-550 cfu and 3000-0 cfu) | *M. abscessus* |
| **NO**<sup>[78]</sup> | *In vitro* study on planktonic and immobilized (agar) bacterial cultures treated with NO (250 ppm/400 ppm) for up to 10 h Antibacterial activity was noted which was independent of pH changes | *M. abscessus* (Multi drug resistant) |
| NO (Novoteris, USA)<sup>[79]</sup> | 8 subjects completed study (2 CF, 3 non-CF bronchiectasis, 3 NTM pulmonary disease) Treatment 160 ppm gNO for 50 min, TID during 5 weekdays for 3 consecutive NTM NTM bacterial loads weeks reduced in 50% of subjects (4/8) by at least two log<sub>10</sub> cfu/gm sputum Complete eradication of NTM in 3/4 subjects and 1/3 had regrowth week 8 post study Preliminary results indicate well tolerated but compared to *in vitro* results, may require a longer regimen to achieve eradication of NTM | *M. abscessus* |
| Orphan drug QRM-003 (Qrumpharma) novel nebulized antibiotic which utilises which utilizes clofazimine as the active pharmaceutical ingredient<sup>[80]</sup> | Models used included in vitro macrophage uptake assay, *in vitro* antibiotic susceptibility testing, and two *in vitro* mouse models of NTM infection - *SCID* mouse infection with *M. abscessus* (MABSC), and Beige mouse infection with *M. avium* (MAC) Potent antimycobacterial activity in vitro *In vivo* administration significantly reduced bacterial recovery in both acute MABSC and MAC infection models Activity was significantly greater than oral administration of the clofazimine alone, despite higher clofazimine dosing via oral administration | *M. abscessus* |
| **ALIS**<sup>[81]</sup> | Biofilm and *in vitro* rat model ALIS effectively penetrated NTM biofilms, enhanced AMK uptake into macrophages, both *in vitro* and *in vivo*, and AMK was retained within airways and lung tissue | *M. avium* biofilms |
| **ALIS**<sup>[82]</sup> | A prospective, open-label, randomized study ALIS in addition to guideline-based therapy for treatment-refractory MAC lung disease achieved significantly greater culture conversion by month 6 than guideline-based therapy alone | Bronchiectasis, COPD patients with MAC |
| **LAI**<sup>[83]</sup> | Treatment of 5 patients (590 mg of LAI (70 mg/mL), single daily dose every day for 3 months and then every other month with clarithromycin continuous treatment) 3 patients completed treatment and did not have any respiratory exacerbation, showed negative sputum cultures for *M. abscessus* and stabilized their spirometric functions | *M. abscessus* |
| **LAI**<sup>[84]</sup> | Phase II randomized trial Addition of LAI may be an effective treatment option for NTM lung disease that is refractory to available multidrug treatment regimens | Patients with MAC or *M. abscessus* disease |

**SCID:** Severe combined immunodeficient, **COPD:** Chronic Obstructive Pulmonary Disease, *M. abscessus:* *Mycobacterium abscessus,* AMK: Amikacin, LAI: Liposomal amikacin for inhalation, LAM: Liposomal amikacin for inhalation, ALIS: Amikacin liposome inhalation suspension, NO: Nitric oxide, MAC: *Mycobacterium avium* complex, NTM: Nontuberculous mycobacteria, MABSC: *Mycobacterium abscessus* complex, *M. avium:* *Mycobacterium avium,* INH: Isoniazid
Table 7: Repurposing of drugs

| Repurposed drug                  | Study findings                                                                 | Species used                                                                 |
|----------------------------------|-------------------------------------------------------------------------------|-------------------------------------------------------------------------------|
| Bedaquiline (ATP synthase inhibitor) [85] | Zebrafish model                                                               | M. abscessus                                                                 |
| Bedaquiline [86]                 | Antimycobacterial activity observed                                          | M. smegmatis                                                                |
|                                  | MIC ranged from 0.015 to >2 ug/ml                                             | M. phlei                                                                    |
|                                  | Bedaquiline had a significantly higher MBC compared to the respective MIC, suggesting a bacteriostatic effect | M. duvalii                                                                 |
|                                  | M. flavescens is naturally resistant to bedaquiline and a high MIC correlates with the mutation found at amino acid 63 in AtpE (alanine replaced by methionine) | M. cosmeticicum                                                             |
|                                  |                                                                               | M. mucogenicum                                                              |
|                                  |                                                                               | M. neoaurum                                                                 |
|                                  |                                                                               | M. peregrinum                                                               |
|                                  |                                                                               | M. parafortuitum                                                            |
|                                  |                                                                               | M. flavescens                                                               |
|                                  |                                                                               | M. fortuitum                                                                |
|                                  |                                                                               | M. mageritense                                                              |
|                                  |                                                                               | M. wolinskyi                                                                |
|                                  |                                                                               | M. abscessus                                                                |
|                                  |                                                                               | M. cheloneae                                                                |
|                                  |                                                                               | M. franklinii                                                               |
| Bedaquiline [87]                 | Isolates from patients with pulmonary NTM                                     | M. avium                                                                    |
|                                  | M. avium (MIC=0.06-0.12 μg/mL)                                                | M. intracellulare                                                          |
|                                  | M. intracellulare (MIC=0.06-0.25 μg/mL)                                        | M. kansasii                                                                 |
|                                  | Moderate in vitro activity was noted                                           | M. abscessus                                                                |
|                                  | M. abscessus and M. massiliense were more susceptible to bedaquiline than     | M. intracellulare                                                          |
|                                  | M. fortuitum, with MIC50 and MIC90 values of 0.13 and >16 mg/L, respectively  | M. kansasii                                                                 |
|                                  |                                                                               | M. abscessus                                                                |
|                                  |                                                                               | M. massiliense                                                             |
|                                  |                                                                               | M. fortuitum                                                                |
| Bedaquiline [88]                 | In vitro broth microdilution susceptibility testing of 103 respiratory isolates | MAC                                                                         |
|                                  | MICs ≤0.008 μg/ml (90% of isolates)                                            | M. abscessus                                                                |
|                                  | 102/103 isolates had MICs of ≤0.015 μg/ml                                     | M. intracellulare                                                          |
| Bedaquiline [89]                 | In vitro susceptibility study of 197 clinical isolates form sputum and bronchoalveolar fluid | M. abscessus                                                                |
|                                  | MIC50 of 0.062 and an MIC90 of 0.125 mg/L                                     | Decreased associated with mutations in mab_4384, the gene encoding the repressor of efflux pump MmpS5/MmpL5 |
| Clofazimine [90]                 | In vitro susceptibility study of 209 clinical and reference isolates          | M. abcessus                                                                |
|                                  | Single-direction cross-resistance between bedaquiline- and clofazimine (Cfz)-resistant isolates was observed | M. fortuitum                                                                |
|                                  |                                                                               | M. kansasii                                                                 |
|                                  |                                                                               | M. avium                                                                    |
|                                  |                                                                               | M. intracellulare                                                          |
| Clofazimine [91]                 | Observational-cohort study assessed clofazimine as used for paediatric and adult CF and non-CF patients with pulmonary and extrapulmonary NTM infection (112 patients) | MABSBC                                                                      |
|                                  | Safe oral drug with antimycobacterial activity                                 | MAC                                                                         |
| Rifabutin [92]                   | MICs 3±2 μM (3 μg/ml)                                                         | M. abscessus Bamboo                                                         |
|                                  | Active against clarithromycin-resistant strains                                | M. abscessus subsp. (abscessus, bolletii, massiliense)                       |
| Apramycin, Framycetin (Veterinary antibiotics) [93] | In vitro antibiotic susceptibility analysis                                     | M. abscessus                                                                |
|                                  | Sensitivity to apramycin and framycetin noted                                  | M. abscessus                                                                |
|                                  | Resistant to other veterinary antibiotics (cefovecin, ceftiofur, lincomycin, pirlimycin, and spectinomycin) | M. abscessus                                                                |
| Non-antibiotic medicines used commonly in CF therapy [94] | In vitro analysis of clinical isolates                                        | M. abscessus                                                                |
|                                  | Antimycobacterial activity with chlorphenamine, cyclidine, ibuprofen, and lamisoprazole | M. abscessus                                                                |
| Zafirlukast, a leukotriene receptor antagonist [95] | Zafirlukast (100 μg/ml) reduced M. abscessus free and total growth in THP-1 derived macrophages by 62% and 94%, respectively and reduced interleukin-8 concentration in supernatants from infected THP-1 derived macrophages by 99% | M. abscessus                                                                |

M. massiliense: Mycobacterium massiliense, M. smegmatis: Mycobacterium smegmatis, M. phlei: Mycobacterium phlei, M. abscessus: Mycobacterium abscessus, M. intracellulare: Mycobacterium intracellulare, M. smegmatis: Mycobacterium smegmatis, M. duvalii: Mycobacterium duvalii, M. cosmeticum: Mycobacterium cosmeticum, M. mucogenicum: Mycobacterium mucogenicum, M. neoaurum: Mycobacterium neoaurum, M. peregrinum: Mycobacterium peregrinum, M. parafortuitum: Mycobacterium parafortuitum, M. flavescens: Mycobacterium flavescens, M. fortuitum: Mycobacterium fortuitum, M. mageritense: Mycobacterium mageritense, M. wolinskyi: Mycobacterium wolinskyi, M. cheloneae: Mycobacterium cheloneae, M. franklinii: Mycobacterium franklinii, M. avium: Mycobacterium avium, MICs: Minimum inhibitory concentrations, CF: Cystic fibrosis, MBC: Minimal bactericidal concentration, NTM: Nontuberculous mycobacteria
Millar and Moore: Novel antibacterial approaches to the NTMs

| Approach | Study findings | Species used |
|----------|----------------|--------------|
| **Screening libraries** | | |
| Natural products from different geographical regions in Africa (AfroDB)⁹⁹ | In silico screening, 3D-modeling, bioactivity and pharmacological profiling identifying novel anti-buruli ulcer substances | M. ulcerans |
| The Pathogen Box (http://www.pathogenbox.org/) and GSK’s small-molecule M. tuberculosis leads⁹⁹ | 17/568 compounds identified a hit³⁷³ | M. abscessus |
| | 11/17 novel compounds against M. abscessus: GW623128X (target MmpL3) GSK2200160A, GSK2200157A (target CTP synthetase/MmpL3) MMV687807 (target proton gradient) MMV688978 (target thiol-redox homeostasis) BRL-7940SA, BRL-10988SA, BRL-8903SA, BRL-10143SA, BRL-51091AM (target DHFR inhibitor) GSK1812410A (target unknown) | |
| | Developed a phenotype-based fluorescence assay for rapid screening of compound libraries against M. abscessus | |
| | MIC values and dose-dependent killing curves were determined using the broth microdilution | |
| | Effective compounds identified using a screening assay with isolates grown in the mid-log phase followed by resazurin reduction assays | M. abscessus |
| | Inhibition of growth was confirmed using a dose-response experiments | |
| | MMV688844 showed the best in vitro activity | |
| | High hits noted amongst TB active compounds | M. abscessus |
| | Most of the top hits had a MIC<10 μM Derivatives included: Oxazolidinone derivatives targeting the ribosome: sutezolid (MMV688756), radezolid (MMV688327) and a synthesis intermediate MMV687146 (an indole-2-carboxamide) and MMV688846 (a piperidinyl), targets of the trehalose monomycolate transporter MmpL3 and disrupt mycolic acid synthesis MMV687730, a benzimidazole that inhibits the assembly of FtsZ in M. tuberculosis by enhancing the GTase activity and destabilizing FtsZ polymer which is essential for growth in NTMs MMV687812 is an aminopyrazinamide which binds specifically to mycobacterial GyrB at its ATPase domain MMV675968, an anti-cryptosporidiosis compound that inhibits dihydrofolate reductase in Pneumocystis carinii and Toxoplasma gondii MMV688845 (GSK1729177A) which target RNA polymerase in M. tuberculosis MMV688844 has been predicted, based on in silico analyses, to target ABC transporters (Rv0194) in M. tuberculosis | M. avium |
| **Drug delivery** | | |
| Silver nanoparticles (AgNPs)¹⁰² | AgNP-VAM conjugate (Silver nano particle/vancomycin conjugate) enhanced internalization of conjugate in M. smegmatis cells compared to bare AGNPs or free VAM | M. smegmatis |
| **Anti-virulence compounds** | | |
| Screening of chemical compound collections¹⁰³ | Dictyostelium discoideum host model | M. marinum |
| **Activation of antibacterial autophagy** | | |
| Cyclic peptides ohmyungsamycins¹⁰⁴ | Drosophila melanogaster- M. marinum model Activation of antibacterial autophagy via AMPK-dependent signaling and suppressing excessive inflammation was demonstrated | M. marinum |
| **Bacteriophages** | | |
| Novel antimycobacterial phages¹⁰⁵ | Detailed analysis a mycobacteriophage PDRPv | M. smegmatis M. tuberculosis |

DHFR: Dihydrofolate reductase, MmpL3: Mycobacterial membrane protein Large 3, M. abscessus: Mycobacterium abscessus, M. smegmatis: Mycobacterium smegmatis, M. avium: Mycobacterium avium, M. marinum: Mycobacterium marinum, MICS: Minimum inhibitory concentrations, GSK: GlaxoSmithKline, MMV: Medicines for Malaria Venture, M. tuberculosis: Mycobacterium tuberculosis, M. ulcerans: Mycobacterium ulcerans, NTM: Nontuberculous mycobacteria, 3D: Three-dimensional, AMPK: Adenosine monophosphate-activated protein kinase, AGNPs: Silver nano particles, VAM: Vancomycin. GSK:
Millar and Moore: Novel antibacterial approaches to the NTMs

Pulmonary disease by non-tuberculous mycobacteria – Clinical

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