Original Article

# Antimicrobial Effect of Dimethyl sulfoxide and *N, N*-Dimethylformamide on *Mycobacterium abscessus*: Implications for Antimicrobial Susceptibility Testing

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## Abstract

**Background:** The emergence of antimicrobial resistance globally has initiated the discovery of novel antibiotics and other antimicrobial substances. Many of these novel compounds may be found in phytochemicals, where these novel compounds are extremely difficult to redissolve for antimicrobial susceptibility testing, following extraction. The aim of this study was to examine the potential antimicrobial effects of the common solvents, dimethyl sulfoxide (DMSO) and *N*, *N*-dimethylformamide (DMF), which are commonly employed as solvents of novel antimicrobial substances, with the nontuberculous *Mycobacterium* and *Mycobacterium abscessus*. **Methods**: *M. abscessus* clinical isolates ($n = 17$ isolates) were examined for the antimicrobial effects of DMSO and DMF. McFarland 0.5 standards of each isolate were prepared individually on Columbia Blood agar onto which DMSO and DMF were added (10 µl) in the range neat (undiluted) – 10,000-fold ($10^{-4}$) dilution and incubated. Zones of inhibition were recorded in mm. **Results**: DMSO and DMF had an inhibitory effect on *M. abscessus* ($n = 17$ clinical isolates). This inhibitory effect was avoided by diluting DMSO 10-fold and DMF 10,000-fold. **Conclusion**: Such data are important when employing these common solvents with molecules which are difficult to dissolve into solution, including conventional antibiotics, as well as novel antimicrobial agents, particularly in antimicrobial susceptibility studies. Investigators should therefore be aware of this inhibition and avoid working with these solvents at high concentration to avoid bacterial growth inhibition. The use of appropriate experimental controls is highly recommended in such circumstances to avoid the reporting of false-positive antimicrobial effects.

**Keywords:** Antimicrobial resistance, antimicrobial susceptibility testing, cystic fibrosis, microbiology, *Mycobacterium abscessus*, nontuberculous mycobacteria

## Introduction

Cystic fibrosis (CF) is a genetic, autosomal recessive disease affecting predominately Caucasian populations of European ancestry, at an approximate rate of 1 in 2500 births.[1] Symptoms of disease relate to the wide tissue distribution of a defective gene product, namely the CF transmembrane conductance regulator (CFTR).[2] Failure or altered expression of this gene product results in abnormal mucous secretions that occlude airway and ductal lumens leading to recurrent pulmonary infections, pancreatic insufficiency, and intestinal obstruction syndromes.

The role of bacterial pathogens in CF pulmonary disease contributes greatly to the morbidity and mortality in patients with CF. Recently, the nontuberculous mycobacteria (NTMs) have emerged as important pathogens in this patient population.[3,4] NTMs are those mycobacterial species outside the *Mycobacterium tuberculosis* group of organisms, particularly the *Mycobacterium avium* complex and the *Mycobacterium abscessus* complex, which are commonly distributed throughout nature and can be isolated from natural water, tap water, and soil.[5] NTMs have been recognized as causing disease in humans since the 1950s,[6] but before 1990, only 16 cases were reported in the literature as being

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linked to CF.[5] Recently, NTMs within CF have emerged globally as a significant clinical problem, where estimates of the prevalence of NTM-positive sputum cultures have ranged from 6% to 30%.[7] These organisms are clinically significant due to their virulence, manifesting in greater morbidity and mortality, associated with a more rapid decline in lung function. Simultaneously, antibiotic treatment of these NTMs is complicated by the high level of multiresistance and panresistance associated with clinical isolates from CF patients. The presence of such high levels of antibiotic resistance in these NTM organisms has thus stimulated research interests in discovering novel sources of antimicrobials, which show activity against these organisms.

The solvents, dimethyl sulfoxide (DMSO), and *N*, *N*-dimethylformamide (DMF) are commonly used to dissolve relatively insoluble anti-infective molecules, including conventional antibiotics such as ethionamide and rifampin, as well as a novel phytochemicals, which have antibacterial activity against *Mycobacterium* spp. However, to date, there have been no reports regarding the inhibitory effects of such solvents on the growth of *M. abscessus*. It was therefore the aim of this study to investigate potential inhibitory effects of DMSO and DMF on clinical isolates of *M. abscessus.*

## Methods

*M. abscessus* clinical isolates ($n$ = 17) were obtained from the HSC Microbiology Culture Repository, MicroARK (www.microark.com), housed at the Northern Ireland Public Health Laboratory, at Belfast City Hospital. All isolates had been historically stored on slopes of Lowenstein–Jensen medium in glass universal containers at ambient temperature. All isolates were recovered and passaged twice on Columbia agar base (Oxoid CM0331; Oxoid Ltd., Basingstoke, UK) supplemented with 5% (v/v) defibrinated horse blood, which was incubated at 37°C for 5 days, before employment in the current study. A fresh culture of each isolate was prepared as described above and was harvested into 0.1% (w/v) peptone saline (CM0733) to yield a 0.5 McFarland inoculation standard. Inoculum was streaked onto fresh Columbia agar base (Oxoid CM0331) supplemented with 5% (v/v) defibrinated horse blood and allowed to dry. Following this, 10 µl DMSO (Sigma Aldrich, UK) and DMF (Fluka Ltd., UK) were added carefully in duplicate onto the surface of the preinoculated plate and allowed to dry, before incubation, as detailed above. In addition, serial dilutions of DMSO and DMF were prepared separately in 0.1% (w/v) peptone saline (CM0733) covering the range 1:10 ($10^{-1}$), 1:100 ($10^{-2}$), 1:1000 ($10^{-3}$), and 1:10,000 ($10^{-4}$) and were added as above. Peptone saline (0.1% w/v) diluent controls were also included. Inhibition was defined as the diameter of no growth (mm) surrounded by confluent mycobacterial growth. Resulting zones of inhibition were compared statistically between DMSO and DMF at all concentrations tested, where $P < 0.05$ (5%) was considered statistically significant.

## Results

Mean inhibition of *M. abscesses* by varying concentrations of DMSO and DMF is shown in Figure 1. No inhibitory effect was seen with 0.1% (w/v) peptone saline diluent. With undiluted DMSO, inhibition of 16/17 (94.1%) isolates was noted, while undiluted DMF inhibited all the isolates. However, when the DMSO was diluted 10-fold (1 log), there was no visible inhibition of any of the 17 isolates. In contrast, the DMF was more inhibitory than the DMSO, as inhibition with DMF was observed through to 1:1000 dilution, with no inhibition being observed at a dilution of 1:10,000.

## Discussion

The emergence of antimicrobial resistance globally has stimulated drug discovery for novel antimicrobial agents or the repurposing of existing drugs. The pipeline of novel antimicrobial molecules may originate from medicinal chemistry, synthetic biology, existing drug repurposing, or from natural sources (phytochemicals) sources. One issue common to each of these sources is the relative inability to find suitable solvent(s), in which to dissolve such novel molecules to enable antimicrobial susceptibility testing. Commonly, such molecules are insoluble in water or aqueous solution, requiring an exploration of suitable organic solvents. DMSO and DMF have been long established solvents used for the solubilization of conventional antibiotics and other difficult-to-dissolve anti-infective molecules. Furthermore, well-established antimicrobial susceptibility testing protocols such as Clinical and Laboratory Standards Institute guidelines recommend the employment of these solvents to dissolve insoluble antibiotics such as ethionamide (DMSO) and rifampin (DMSO or DMF) with *Mycobacterium* spp.[8] Therefore, it is important to examine any potential antimicrobial effect *per se* originating from such DMSO and DMF solvents.

Overall, while DMSO and DMF are good solvents to allow solubilization of conventional antibiotics and novel

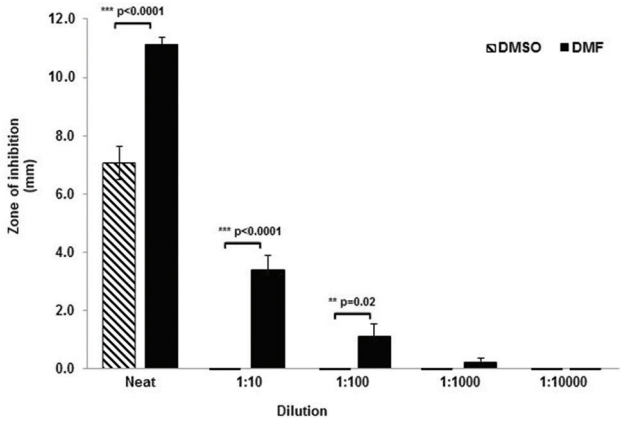


**Figure 1**: Effect of varying concentrations of dimethyl sulfoxide and *N*, *N*-dimethylformamide on growth inhibition of clinical isolates of *Mycobacterium abscessus* ($n$ = 17). Error bars represent standard error of the mean

anti-infective molecules under antimicrobial investigation, employment of such solvents is not without risk of inhibiting the bacterial species being examined and the potential reporting of false-positive effects. Researchers employing such solvents should therefore always include an appropriate control in their investigations to detect potential inhibition of bacterial growth.

In conclusion, from this study and when working with *M. abscessus*, DMSO should not be used undiluted and should be diluted by at least 1:10 (10-fold), whereas DMF should also not be used undiluted but should be diluted by at least 1:10,000 (10,000-fold) to avoid inhibition of organisms from such solvents.

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## Conflicts of interest

There are no conflicts of interest.

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