**In vitro Activity of Seven Hospital Biocides against**

*Mycobacterium abscessus*: Implications for Patients with Cystic Fibrosis

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**Abstract**

**Background:** *Mycobacterium abscessus* pulmonary infection has recently emerged as a significant pathogen in patients with cystic fibrosis (CF) and is associated with significant morbidity and accelerated pulmonary decline. There is a paucity of data describing the activity of hospital biocides against this organism. **Methods:** *M. abscessus* isolates (*n* = 13) were recovered from CF and non-CF respiratory specimens. Seven commonly employed hospital biocides with generic ingredients as follows: acetone, propan-2-ol, diethylene glycol, 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one, chlorine dioxide, 4% chlorhexidine, alcohol, and disodium carbonate, compound with hydrogen peroxide, 10% sodium hypochlorite were assayed for their biocidal activity against *M. abscessus*. Fresh cultures of *M. abscessus* were exposed to biocide in liquid medium as per manufacturers’ instruction and were immediately plated following the completion of the contact period. The mean concentration of *M. abscessus* plated was 9.82 × 10⁶ colony-forming units (range: 1.63 × 10⁵–1.12 × 10⁷). In addition, the remaining bacteria/biocide solution was enriched nonselectively in Mueller Hinton broth (37°C/1 week) and then plated. **Results:** All *M. abscessus* isolates survived in alkyl dimethyl benzyl ammonium chloride, 5-chloro-2-methyl-2H-isothiazol-3-one (EC No. 247-500-7) and 2-methyl-2H-isothiazol-3-one, 4% Chlorhexidine™, O-phenylphenol and Sodium Lauryl Sulfate™ and disodium carbonate, compound with hydrogen peroxide. One out of 13 *M. abscessus* cultures was killed by Chlorine Dioxide™ and one by Sodium Dichloroisocyanurate™, representing a 5-log kill. Two isolates were killed by Alcohol™ again representing a 5 log kill. Following enrichment, O-phenylphenol and Sodium Lauryl Sulfate™ showed the greatest biocidal activity with 11/13 isolates, whereas 2/13 cultures were killed by sodium dichloroisocyanurate™. All other biocide/culture combinations yielded growth. **Conclusion:** These data indicate that *M. abscessus* may persist after exposure to several common hospital biocides. Further work is urgently needed to define unequivocal biocide contact treatments to prevent cross-infection with this mycobacterial species in this patient population and thus ensure effective infection control and prevention.

**Keywords:** Biocide, cystic fibrosis, disinfection, *Mycobacterium abscessus*, nontuberculosis mycobacteria

**INTRODUCTION**

Cystic fibrosis (CF) is the most common life-limiting, autosomal recessive disease worldwide and is caused by mutations in the CF transmembrane conductance regulator (CFTR) gene. The subsequent defective CFTR protein results in abnormalities in both salt and fluid transport across epithelia. In the lung, this leads to dehydration of the airway surface and impaired mucociliary clearance. This failure of innate defense and subsequent retention of mucus provides an environment in which opportunistic pathogens such as *Pseudomonas aeruginosa, Staphylococcus aureus*, and *Burkholderia cepacia* complex may cause chronic infection. Nontuberculous mycobacteria (NTM) also have been shown to cause chronic lung infections in patients with CF. The NTM species most commonly cultured in patients with CF in Europe and the USA are the slow-growing *Mycobacterium avium* complex (including *M. avium, Mycobacterium intracellulare*, and *Mycobacterium chimaera*), which can be found in approximately 60%–70% of NTM-positive sputum samples.
cultures\textsuperscript{[2,2]} and the rapidly growing *Mycobacterium abscessus* complex including *M. abscessus* spp *M. a. bolletii*\textsuperscript{[4,4]} and *M. a. massiliense*.\textsuperscript{[5–7]} Of the rapidly growing NTM species, *M. abscessus* has emerged as a major respiratory pathogen in individuals with CF.\textsuperscript{[7,7]} *M. abscessus* is a ubiquitous, highly resistant, rapidly growing NTM, which is pathogenic in CF and can result in accelerated pulmonary decline.\textsuperscript{[8]} *M. abscessus* affects between 3% and 10% of patients with CF in the USA and Europe, and worryingly, the prevalence is increasing.\textsuperscript{[2,7,9]} The reasons for the apparent increase in *M. abscessus* infections in patients with CF remain unclear. Suggested reasons include (i) increases in environmental exposure to NTM through more permissive temperature settings of home water heaters;\textsuperscript{[10]} (ii) increasing contact with aerosols from contaminated showerheads;\textsuperscript{[11,12]} (iii) the establishment of permissive lung niches through increased inhaled antibiotic usage;\textsuperscript{[13]} (iv) impairment of host autophagy inhibition by chronic azithromycin therapy, and (v) spread of NTM through person-to-person transmission.\textsuperscript{[7,14,15]} Treatment regimens are frequently toxic and prolonged and are therefore often poorly tolerated or unsuccessful. Acquisition of *M. abscessus* may also preclude safe lung transplantation.\textsuperscript{[16]}

Traditionally, acquisition of *M. abscessus* has been attributed to environmental contacts such as contaminated soil or water sources although the potential for direct and indirect transmission has been highlighted recently.\textsuperscript{[7]} The CF Foundation recommends specific infection control measures to reduce the incidence of patient-to-patient transmission. Health-care personnel are recommended to perform hand hygiene (either using alcohol-based hand rub or washing hands with antimicrobial soap and water), as per the Centers for Disease Control and Prevention (CDC)\textsuperscript{[17]} and UK CF Trust guidelines.\textsuperscript{[18]} The CF Foundation also recommends that examination rooms be cleaned and disinfected between patients using a one-step process and an Environmental Protection Agency (EPA)-registered hospital-grade disinfectant/detergent designed for housekeeping in accordance with institutional infection prevention and control policies.\textsuperscript{[19]} Despite these recommendations, recent studies have confirmed *M. abscessus* outbreaks within the CF population.\textsuperscript{[1,20]} Data on the outcomes of *M. abscessus* outbreak and the efficacy of specific hospital biocides remains limited.

Therefore, it was the aim of this study to compare the activity of commonly used hospital biocides against *M. abscessus*.

**Methods**

*M. abscessus* isolates \((n = 13)\) were recovered from CF and non-CF respiratory specimens. Seven commonly employed hospital biocides were selected for evaluation (generic constituents), as follows:

i. Acetone
ii. Propan-2-ol
iii. Diethylene glycol
iv. 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one
v. Chlorine dioxide
vi. 4% chlorhexidine
vii. Alcohol
viii. Disodium carbonate, compound with hydrogen peroxide, 10% sodium hypochlorite.

These formulations were prepared in liquid form in concentrations according to their specific manufacturers’ instructions and then assayed in vitro, for their biocidal activity against *M. abscessus*. Fresh cultures \((t = 48 \text{ h})\) of *M. abscessus* were exposed to biocide in for the duration of the manufacturer’s prescribed contact period and were then immediately plated onto Columbia blood agar (Oxoid CM0331, Oxoid UK Ltd., Basingstoke, UK) supplemented with defibrinated horse \((5\%\ v/v)\). The mean concentration of *M. abscessus* isolates plated was \(9.82 \times 10^6\) colony-forming units \((\text{range: } 1.63 \times 10^6 – 1.12 \times 10^8)\). In addition, the remaining mycobacteria/biode solution were enriched nonselectively in Mueller Hinton broth (Oxoid CM0405) at 37°C for a further 1-week period and then plated to assess for growth of surviving organisms, as described above.

**Results**

After appropriate exposure of *M. abscessus* isolates to each biocide, all *M. abscessus* isolates were shown to survive treatment with alkyl dimethyl benzyl ammonium chloride, 5-chloro-2-methyl-2H-isothiazol-3-one (EC No 247-500-7), and 2-methyl-2H-isothiazol-3-one, 4% chlorhexidine, o-phenylphenol and sodium lauryl sulfate, and disodium carbonate, compound with hydrogen peroxide. One out of 13 *M. abscessus* isolates was killed by chlorine dioxide and one isolate was killed by sodium dichloroisocyanurate, both cases representing a 5-log kill. Two isolates were killed by alcohol, again representing a 5-log kill. Following a 1-week period of enrichment, O-phenylphenol and Sodium Lauryl Sulfate\textsuperscript{TM} showed the greatest antimycobacterial activity killing 11 of the 13 *M. abscessus* isolates, whereas 2 of the 13 cultures were killed by sodium dichloroisocyanurate. All other biocide/culture combinations yielded growth.

**Discussion**

Data regarding the efficacy of specific infection control measures employed to limit cross-infection with this organism remain sparse. CF infection control guidelines highlight the significance of *M. abscessus* infection and recommend strict infection control measures to minimize the potential for direct or indirect transmission.\textsuperscript{[19,20]} Measures such as advising patients not to mix socially and the use of individual rooms for inpatient treatment and for outpatient clinic review are recommended. Hand hygiene with either using alcohol-based hand rub or washing hands with antimicrobial soap and water, as per the CDC and WHO guidelines is recommended. The CF Foundation also recommends that examination rooms be cleaned and disinfected between patients using an
EPA-registered hospital-grade disinfectant/detergent designed for housekeeping.

In this study, we examined the ability of seven commonly employed biocides to eliminate CF and non-CF clinical isolates of *M. abscessus*. None of the biocides examined was able to totally eliminate all the clinical isolates of *M. abscessus* tested. The concerning finding of *in vitro* resistance to common hospital biocides used for handwashing and environmental sterilization also raises several important concerns about the effectiveness of current infection control measures employed in CF treatment centers and the potential for direct or indirect spread of *M. abscessus*.

Coupled with this, patients with CF are often in frequent contact with clinical environments, further increasing their risk of acquisition of *M. abscessus* infection. Modern CF units are now generally recommended to consider providing negative pressure inpatient and outpatient rooms to diminish the risk of airborne contamination in communal areas outside the rooms. This may not be the case in older treatment centers. Transmission can occur from patients with persistently smear-negative, culture-positive sputum, suggesting that the inoculum needed for successful infection could be low. Alternatively, aerosol generation during physiotherapy and lung function testing could lead to cross-infection through inhalation of airborne water droplets, from which NTM have been cultured in the environment.

Given the importance of effective infection prevention and control, further work is urgently needed to define unequivocal biocide contact treatments to ensure clinical environments are safe for patients with CF.

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**Conflicts of interest**

There are no conflicts of interest.

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