**In Vitro Antimicrobial Efficacy of a Silver-Containing Wound Dressing Against Mycobacteria Associated with Atypical Skin Ulcers**

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**Abstract:** *Mycobacterium ulcerans*, the etiological agent in Buruli and related ulcers, is a major threat to public health in many tropical countries. Recommended treatment that is accessible and affordable for affected individuals includes surgical debridement and combination antibiotics. The potential benefits in the use of antimicrobial wound dressings has not been demonstrated to date, and consequently the efficacy of a silver-containing absorbent dressing was investigated against a pathogenic wound mycobacterium using stringent *in vitro* models. The *in vitro* models were designed to simulate a variety of challenging wound conditions. *Mycobacterium fortuitum* was used as a fast-growing surrogate for *M. ulcerans*, a physiologically similar but slower-growing and more significant wound pathogen. Collectively, the studies showed that the silver-containing dressing was bactericidal against *M. fortuitum*, it maintained killing effect over a prolonged period (7 days) under conditions simulating excessive exudate, and killed an average of 100% of the bacterial population inoculated directly beneath the dressing in a simulated, colonized, shallow wound model. Based on the *in vitro* data generated in the current research, use of the silver-containing dressing as part of a protocol-of-care in the management of Buruli and related ulcers may help to alleviate wound infection caused by pathogenic mycobacteria, improve quality of life, and provide infection protection in endemic and at-risk regions.

**Key words:** mycobacteria, Buruli ulcer, antimicrobial wound dressing

A typical skin wounds caused by some of the *Mycobacterium* species are generally rare but are a significant threat to public health, particularly in some tropical and subtropical countries. Skin and soft tissue infections caused by *M. abscessus* are increasingly recognized,^{1,2} sporadic cases of skin ulcers caused by *M. tuberculosis* and *M. chelonae* have been reported,^{3,4} and neuropathic ulcers are frequently associated with *M. leprae.*^{5} However, ulcers caused by *M. ulcerans* are more widespread, particularly in West Africa, Australia, Southeast Asia, China, Central and South America, and the Western Pacific.^{6} Buruli, Bairnsdale, Daintree,
Kumusi, and Searles are all local names for the same type of skin ulcer that is caused by *M. ulcerans*. Mycobacteria are distinct among bacteria in that they possess a thick, lipid-rich and hydrophobic cell envelope that provides resistance to desiccation and environmental tolerance. *M. ulcerans* is typical in this respect and, additionally, it is a slow-growing species (with associated skin ulcers developing over many months to years) and grows optimally at 30°C-33°C. Infection typically occurs following trauma to the skin and entry of the pathogen from waterborne sources, including aquatic insects. Production of mycolactone by *M. ulcerans* is subsequently responsible for local immunosuppression, tissue destruction, and subcutaneous necrosis leading to ulceration with undermined edges. The Buruli ulcer is recognized as the third most common mycobacterial infection after tuberculosis and leprosy, and the potentially devastating clinical complications and often unaffordable treatment costs create a huge socioeconomic problem.

Treatment options for mycobacteria are limited as a consequence of their intrinsic resistance to antimicrobial agents; this is attributed in part to the low permeability of their unique cell envelope and the low metabolic activity and slow-growing nature of many species. The World Health Organization recommends a combination of rifampin and streptomycin/amikacin for 8 weeks together with surgery to remove necrotic tissue and to cover skin defects as a first-line treatment for Buruli ulcers. Additionally, topical therapies such as raising the local tissue temperature to above optimum (ie, 40°C) for *M. ulcerans*, and application of nitrogen oxide-releasing creams, have produced favorable outcomes. To date, there has been no formal consideration of the potential benefits of antimicrobial wound dressings in the control of *M. ulcerans* and management of Buruli ulcers.

Antimicrobial dressings are widely used in the management of chronic and acute wounds that are infected or at risk of infection. Over the past decade, silver has been the topical antimicrobial agent of choice and is available in a wide variety of dressing types. One such dressing type is based on Hydrofiber (ConvaTec Inc, Skillman, NJ) technology, which has unique exudate absorption and handling capacity. Following absorption of exudate, the cellulose fibers within the dressing swell to form a cohesive gel that conforms closely to a wound’s topography and locks exudate and bacteria within its matrix. The incorporation of ionic silver into the dressing provides added antimicrobial protection, its efficacy has been demonstrated in vitro, and bioburden reduction and wound progression have been reported in a variety of chronic wounds. Presently, and to the author’s knowledge, no assessment has been made of the efficacy of any commercially available antimicrobial dressing against atypical wound pathogens that can cause serious and debilitating chronic wounds. The objective of this study was to investigate the antimicrobial potential of a silver-containing Hydrofiber dressing against mycobacteria that are associated with atypical skin ulcers. Due to the slow-growing nature and practical difficulties in working with *M. ulcerans*, *M. fortuitum* was used as a surrogate mycobacterium. *M. fortuitum* is physiologically similar to *M. ulcerans* (ie, it has a thick and low-permeable cell envelope), it has been associated with skin abscesses and postoperative wound infections, and it is easier to culture due to its fast-growing nature (2 to 4 days).

**Methods**

This study used 3 *in vitro* models that simulated a variety of wound conditions to stringently assess the antimicrobial efficacy of a silver-containing Hydrofiber dressing, dressing A (Aquacel Ag dressing) against *M. fortuitum* (NCTC 8697). The same Hydrofiber dressing without silver, dressing B (Aquacel dressing) was used as a control where appropriate. A simulated wound fluid model (SWF) was used to quantify the killing capacity of the dressing over time under conditions that mimicked an excess of wound exudate. A simulated colonized shallow wound model was used to investigate the impact of dressing conformability in providing antimicrobial activity, and a third model investigated the efficacy of the dressing on bacteria embedded within a simulated colonized wound surface.

*Simulated wound fluid model.* Five (5) cm x 5cm pieces of dressing A were aseptically transferred to 10 ml volumes of SWF (peptone saline diluents and foetal calf serum) containing approximately 1 x 10⁵ cfu/ml of
M. fortuitum. All test models were incubated at 35°C in a 5% CO₂ atmosphere, and total viable counts (TVCs) were performed at several time points over a 7-day test period, using 0.1% sodium thioglycollate to neutralize residual silver activity. On day 2, each test model was reinoculated with approximately 1 x 10⁵ cfu of fresh M. fortuitum to mimic a worst-case clinical situation (ie, to assess whether the dressing could maintain killing capacity despite heavy microbial challenge during the test period). M. fortuitum was tested against dressing A on 5 occasions and against dressing B (control) on 1 occasion (as the control was not expected to provide any antimicrobial effect).

**Simulated colonized shallow wound model.** A sterile layer (80 ml) of MBA in a 140 mm Petri dish was flooded with a second layer of molten agar (45ml) that had previously been seeded with ~1 x 10⁵ cfu/ml of M. fortuitum, to create a seeded agar layer ~2 mm in depth. Following incubation at 35°C in a 5% CO₂ atmosphere for 4 hours to initiate growth, 10 cm x 10 cm pieces of dressing A were applied to the center of each seeded agar plate and pressed down gently to ensure good contact with the agar surface before the application of a secondary adhesive cover dressing (as stated in manufacturer’s instructions for use). Seeded agar plates were then incubated at 35°C in a 5% CO₂ atmosphere for 48 hours, following which the dressings were aseptically removed and photographs of the agar plates were taken. Agar plates (with dressings removed) were then reincubated for an additional 96 hours to enable clear visualization of any remaining viable cells. To assess bacteriostatic and/or bactericidal activity against M. fortuitum, a stab culture was taken from the center of each seeded agar plate using a sterile loop and then sub-cultured on to DE Neutralizing agar to neutralize any residual silver activity. Following incubation under appropriate atmospheric conditions for 72 hours, the sub-cultured agar plates were observed for the presence or absence of bacterial growth. M. fortuitum was tested against dressing A on 5 occasions, and on 1 occasion in the absence of the dressing (control).

**Simulated colonized wound surface model.** A sterile layer (80 ml) of MBA in a 140 mm Petri dish was flooded with a second layer of molten agar (45ml) that had previously been seeded with ~1 x 10⁵ cfu/ml of M. fortuitum, to create a seeded agar layer ~2 mm in depth. Following incubation at 35°C in a 5% CO₂ atmosphere for 4 hours to initiate growth, 10 cm x 10 cm pieces of dressing A were applied to the center of each seeded agar plate and pressed down gently to ensure good contact with the agar surface before the application of a secondary adhesive cover dressing (as stated in manufacturer’s instructions for use). Seeded agar plates were then incubated at 35°C in a 5% CO₂ atmosphere for 48 hours, following which the dressings were aseptically removed and photographs of the agar plates were taken. Agar plates (with dressings removed) were then reincubated for an additional 96 hours to enable clear visualization of any remaining viable cells. To assess bacteriostatic and/or bactericidal activity against M. fortuitum, a stab culture was taken from the center of each seeded agar plate using a sterile loop and then sub-cultured on to DE Neutralizing agar to neutralize any residual silver activity. Following incubation under appropriate atmospheric conditions for 72 hours, the sub-cultured agar plates were observed for the presence or absence of bacterial growth. M. fortuitum was tested against dressing A on 5 occasions, and on 1 occasion in the absence of the dressing (control).
Results

Simulated wound fluid model. Dressing A demonstrated sustained killing of *M. fortuitum*, with an approximate 10,000-fold reduction in bacterial load over the 7-day test period, and despite reinoculation of a further viable population of *M. fortuitum* into the test model on day 2 (Figure 1). Dressing B did not exhibit any killing effect in this model.

Simulated colonized shallow wound model. In this *in vitro* model, dressing A (n = 5) completely killed the *M. fortuitum* inoculum within the simulated shallow wound area beneath the dressing (ie, no growth of *M. fortuitum* in the inoculated simulated wound area) (Table 1). The control agar plate (ie, no dressing applied) demonstrated complete (100% surface area) growth within the inoculated shallow wound area. Examples of the measured antimicrobial activity of dressing A on *M. fortuitum* compared to a control (without dressing) are shown in Figures 2A and 2B, respectively.

Simulated colonized wound surface model. In this *in vitro* model, dressing A (n = 5) demonstrated bactericidal activity in the 2 mm *M. fortuitum*-seeded agar layer, as confirmed by sampling of the clear/nonturbid agar directly beneath the dressing, and further subculture (Table 2). A visual example of the *M. fortuitum* killing capacity in this model is demonstrated in Figure 3A; Figure 3B demonstrates the growth of *M. fortuitum* in the absence of the antimicrobial dressing.

Discussion

Bacterial infections present a major health problem, particularly in underdeveloped countries where large populations and land mass, poor economy, and limited medical care exist. The Buruli ulcer is one such disease that is considered to be neglected, despite the often severe and debilitating clinical consequences such as amputations, contractual deformities, scarring, and disabilities.

Costly combination antibiotic therapy of variable efficacy is the only recognized chemotherapeutic approach for Buruli ulcers, and no commercially available, evidence-based topical products are routinely considered as part of a protocol-of-care. The causative agent of Buruli ulcers, *Mycobacterium ulcerans*, belongs to a unique bacterial genus that is intrinsically resistant to many antimicrobial agents, and this makes the identification of additional combative products even more difficult. The objective of the current research was to assist in identifying treatments that may improve quality of life for the thousands of underprivileged victims who suffer from Buruli or related ulcers.

### Table 1. Simulated colonized shallow wound model. Percentage growth of *M. fortuitum* within the simulated shallow wound area beneath dressing A and in the absence of dressing over a 48-hour contact period.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Challenge Organism</th>
<th>% Bacterial Growth (of surface area within agar indentation)</th>
<th>Mean % Bacterial Growth (within agar indentation)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Replicates</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Dressing A</td>
<td><em>M. fortuitum</em></td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>No dressing (control)</td>
<td><em>(NCTC 8697)</em></td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2. Simulated colonized wound surface model. Bacteriostatic/Bactericidal Activity of the dressing A.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Organism</th>
<th>Activity (visual observation in seeded agar beneath dressing)</th>
<th>Activity (growth/no growth from stab culture from center of seeded agar beneath dressing)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dressing A (n = 5)</td>
<td><em>M. fortuitum</em></td>
<td>No visible growth</td>
<td>No growth</td>
</tr>
<tr>
<td>No dressing (control) (n = 1)</td>
<td><em>(NCTC 8697)</em></td>
<td>Visible growth</td>
<td>Growth</td>
</tr>
</tbody>
</table>

**KEYPOINTS**

This study used 3 *in vitro* models that simulated a variety of wound conditions to stringently assess the antimicrobial efficacy of a silver-containing Hydrofiber dressing, dressing A (Aquacel Ag dressing) against *M. fortuitum* (NCTC 8697).
the current research has been shown to effectively manage fluid and kill a wide spectrum of microorganisms in in vitro models. Good patient outcomes have also been demonstrated in clinical studies involving chronic and acute wounds. While this antimicrobial dressing is known to kill a wide spectrum of common wound pathogens, prior to the current study, it had never been evaluated against intrinsically hardy pathogens that cause atypical chronic ulcers. Consequently, in vitro models simulating challenging wound conditions were used to evaluate the efficacy of this dressing (dressing A) against M. fortuitum. Collectively, the studies showed dressing A was bactericidal against M. fortuitum, it maintained killing effect over a prolonged period (7 days) under conditions simulating excessive exudate; and killed an average of 100% of the bacterial population inoculated directly beneath the dressing in a simulated colonized shallow wound model. Although a wide variety of silver-containing dressings are used in wound management today, their antimicrobial efficacy is variable. This may be influenced by the dressing technology and by the extent to which it is able to make the antimicrobial agent accessible to microorganisms, as has been reported in in vitro studies.

While it is acknowledged that M. ulcerans was not tested in this study, the surrogate bacterium, M. fortuitum is recognized as an atypical wound pathogen in its own right and possesses similar intrinsic resistance factors. It is also recognized that M. ulcerans, like other invading bacteria, may inhabit subcutaneous tissue that is less accessible to topical antimicrobial agents. Although efficacy of dressing A was observed in a 2 mm depth of agar seeded with M. fortuitum, it is evident that further studies are warranted to demonstrate a clinical benefit. Access to, and affordability of, effective therapeutic strategies for controlling Buruli and related ulcers are clearly critical factors in providing improved care to affected patients, and consequently the cost effectiveness of dressing A in this situation needs to be demonstrated.

**Conclusion**

Based on the in vitro data generated in the current research, use of a silver-containing Hydrofiber dressing as part of a protocol-of-care in the management of Buruli ulcers and other atypical wounds involving mycobacteria, may help to alleviate wound infections caused by these unusual pathogens, improve quality of life and provide infection protection in endemic and at-risk regions.

**References**


**Key Points**

The studies using 2 in vitro models showed dressing A was:

- bactericidal against M. fortuitum;
- maintained killing effect over a prolonged period (7 days) under conditions simulating excessive exudate; and
- killed an average of 100% of the bacterial population inoculated directly beneath the dressing in a simulated colonized shallow wound model.


