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Chemical Disinfection of *Mycobacterium ulcerans*

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Mycobacterium ulcerans is the causative agent of Buruli ulcer, a disfiguring disease of the skin and muscle tissue, whose prevalence has grown exponentially in recent decades. An ulcerative disease similar to leprosy, Buruli ulcer is characterized by a superficial infection of the subcutaneous fat layer that results in large lesions that often spread to cover an entire limb. Although most severe in Third World countries, cases of *M. ulcerans* infection have been reported in 33 countries on five continents. According to World Health Organization statistics, the recent rise in incidence of the disease has been so pronounced that Buruli ulcer now threatens to surpass leprosy as the most widespread ulcerative disease worldwide. While much basic research is underway in an effort to better understand this relatively new organism, one particularly important piece of data remains unknown.

To date, no antimicrobial treatment has been effective in a live infection, leaving surgical excision of the lesion as the only effective method of treatment. Such procedures require chemical sterilization of operating surfaces and instruments, which is limited by the fact that mycobacterial species are more resistant to chemical disinfection than most other pathogens. While the resistance of other mycobacterial species such as leprosy and tuberculosis to common surgical disinfectants has been evaluated in previous studies, the susceptibility of *M. ulcerans* to standard chemical disinfectants has not been determined. In addition, one of the mycobacterial species, *Mycobacterium bovis*, has been selected as a challenge organism for disinfectant testing by virtue of its high resistance to chemical agents. If the resistance of *M. ulcerans* was found to exceed that of the currently accepted challenge organism, it would potentially represent a more accurate test subject, which would be very important in the area of anti-mycobacterial testing.

Our research project was designed to answer both of these questions. To begin, we used clinical isolates retrieved from Ghana, West Africa by a team of local surgeons and student volunteers from BYU. It was necessary to culture the isolates in liquid suspension in order to prepare for the testing. To do this and other steps, we followed the U.S. Environmental Protection Agency Tuberculocidal Activity Test Method, an accepted protocol published in current literature. The challenge organism, *M. bovis*, was grown according to identical procedure. The Mycobacterial species in general are among the slowest growing bacteria, and in our study this proved to be a major obstacle. While the challenge bacterium grew as described in the literature, reaching the established concentration, determined by turbidity, in approximately 18 days, *M. ulcerans* failed to proliferate in liquid culture. We searched the available literature and determined that it would be necessary to increase the incubation temperature and decrease the pH of the liquid media. Following these alterations, we were able to grow a suspension to the prescribed turbidity. Following this step, we froze both suspensions in 1 mL portions.

On the day of the initial disinfectant testing, both samples were thawed and then tested for viability by exposing them to a standard disinfectant, 0.8% phenol, for a set amount of time. Following this exposure, both samples were plated at different concentrations on solid media using a technique known as membrane filtration. These plates were then allowed to incubate.

Again, the amount of time required by these organisms to grow became a stumbling block to our project. The *M. bovis* incubated again in approximately 20 days, at which time we were able to count the colonies on each plate, and determine the amount of bacteria that were killed by comparing these plates to a control plate which was not exposed to the disinfectant. However, this reduction value did not fall within the range required for viability of a test organism. As a result, we had to start again, and repeat the process three times more until we achieved useful results.

From the current literature and from correspondence with other researchers working with *M. ulcerans*, we postulated that incubation would take approximately six weeks. The great length of time required for growth explained why there was not more work done on this particular organism, as was evidenced by the relative lack of papers on the subject in scientific journals. To our surprise and disappointment, our cultures required nearly three months to incubate to the point where we were able to count the miniscule colonies under magnification. In order to proceed with further antimicrobial testing, the titer, or concentration of organisms per milliliter of unexposed control media, had to be above a certain value according to the EPA protocol, and, like for the test organism, the concentration post-exposure to the standardized disinfectant had to fall within the range given in the protocol. On our first lengthy attempt, our assay failed in both areas. This came as a major disappointment because of the amount of time required to re-culture the organism. Four months later we were able to count the results of a second attempt, which were both within viability limits and of sufficiently high titer.

At this point, it became clear that in order to test disinfectants, a major time investment would be required. We are currently preparing for this phase of the project, and will hopefully have results for other disinfectants, such as the gold standard surgical disinfectant, orthophthalaldehyde. Initial results from the viability test indicate that *M. ulcerans* is quite resistant, although the extent of the resistance would be more accurately determined when tested against other disinfectants. This forthcoming data, should the planned tests work will provide essential information for field clinicians and basic researchers alike, and will hopefully assist in testing against other pathogens, such as tuberculosis and leprosy.

As this project continues, it becomes apparent that ways to accelerate the incubation process of *M. ulcerans* would greatly improve both the quality and quantity of research that can be performed on this disease and the organism that causes it. At the same time, it is much too early to give up the search for effective antimicrobial treatments for humans, as this would be a much more efficient method of controlling what is becoming a major health issue.