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Review

# Fighting mycobacterial infections by antibiotics, phytochemicals and vaccines

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**Abstract**

Buruli ulcer is a neglected disease caused by *Mycobacterium ulcerans* and represents the world's third most common mycobacterial infection. It produces the polyketide toxins, mycolactones A, B, C and D, which induce apoptosis and necrosis. Clinical symptoms are subcutaneous nodules, papules, plaques and ulcerating oedemae, which can enlarge and destroy nerves and blood vessels and even invade bones by lymphatic or haematogenous spread (osteomyelitis). Patients usually do not suffer from pain or systematic inflammation. Surgery is the treatment of choice, although recurrence is common and wide surgical excisions including healthy tissues result in significant morbidity. Antibiotic therapy with rifamycins, aminoglycosides, macrolides and quinolones also improves cure rates. Still less exploited treatment options are phytochemicals from medicinal plants used in affected countries. Vaccination against Buruli ulcer is still in its infancy.


*Keywords:* Buruli ulcer; Antibiotics; Natural products; Phytochemicals; Vaccination; Neglected diseases

## 1. Epidemiology and transmission

Mycobacterial infections are caused by different species of the genus *Mycobacterium* [1,2]. Mycobacteria exist as environmental saprophytes [3] and are responsible for illness and death all over the world [4]. Non-tuberculous mycobacteria (NTM) also represent important environmental pathogens causing a wide spectrum of diseases [5] with increasing incidence among both immunosuppressed and non-immunosuppressed subjects [4]. Of NTM diseases, Buruli ulcer shows greatest public health menace.

Buruli ulcer, a skin disease caused by *Mycobacterium ulcerans*, represents the world's third most common mycobacterial infection after tuberculosis and leprosy and is the least understood of these three diseases [6,7]. Buruli ulcer was first documented in Australia in 1948 [8,9]. During the 1960s the disease was first diagnosed at Buruli County near the Nile River in Uganda. Subsequently, the disease became more generally known as Buruli ulcer [10,11].

Buruli ulcer has been reported in 30 tropical and subtropical countries worldwide, mostly near rural riverine areas [12,13]. The largest number of Buruli ulcer patients has been found in West Africa. Other cases have been reported in Australia, New Guinea, Malaysia, Bolivia, Mexico, Peru, and China [14,15]. Individuals of all ages can be infected, but the majority of patients is between 5 and 15 years of age, irrespective of sex and race [16,17].

The way of transmission to humans is scarcely known [18]. As most people affected with Buruli ulcer live near lakes, rivers and swamps, the most plausible mode is skin infection by contaminated water, soil or aquatic vegetation [14,19]. Another mode is inhalation of aerosols from water surfaces. Since the DNA of *M. ulcerans* has been detected in water bugs, aquatic insects have also been discussed as transmission vectors [12,20]. Human to human transmission can occur, but does not seem to be a major infection route [16]. The changing epidemiology of Buruli ulcer has been linked to flooding, deforestation, or the introduction of dams and irrigation

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system [18]. Buruli ulcer has also been associated with seasonal variations. For example, there seems to be a relationship between dry periods and Buruli ulcer incidence. Because the mode of transmission is unclear, preventive measures are difficult to realize. The idea of avoiding contact with environmental sources of *M. ulcerans* is difficult. Wearing trousers, boots and long shirts during farming and stays in swamps seem to be useful for protection. Other simple preventive steps are the use of mosquito nets or soap for washing [12,21].

## 2. Pathogenesis

*M. ulcerans* is unique among mycobacteria, because it produces polyketide toxins, called mycolactones [22,23]. Four mycolactones, A, B, C and D, have been characterized by mass spectronomy [22,24]. They are labelled according to their locations [25]. Mycolactones A and B are found in African *M. ulcerans* and are the most common and active ones. They reveal great cytotoxic activity and induce apoptosis and necrosis in several cell types, including monocytes, macrophages, neutrophils, lymphocytes, fibroblasts and dendritic, epithelial and adipose cells. Tissue necrosis and immunosuppression caused by mycolactones may explain missing host symptoms, such as fever [26–28]. The mechanism of action of immunosuppression in *M. ulcerans* infections is unknown. It can be assumed as consequence of the cytotoxicity of mycolactones towards immune cells and the inhibition of cellular inflammation signals (TNF, IL-1β, IL-6, IL-10 and INF-γ) [25,29].

## 3. Clinical features and diagnosis

The early clinical symptoms of Buruli ulcer are subcutaneous nodules, papules, plaques or oedemae, which ulcerate and show characteristic undermined edges [6]. These ulcerations can enlarge and destroy nerves and blood vessels. In some cases, the ulcerations invade the bones (osteomyelitis) by lymphatic or haematogenous spread [12,29]. Sometimes lesions spontaneously heal by unknown mechanisms. Cure from the disease is time-consuming and can cause scars and deformities [6,30]. The patients usually do not suffer from pain or systematic inflammatory responses [16]. The burden of the disease, *i.e.* morbidity, functional disability and socio-economic constrain is high, although the mortality rate is low [31,32].

Clinical diagnosis of Buruli ulcer is usually easy to be done for experienced health professionals in endemic areas. In regions with low incidence and less experience among physicians, the diagnosis should be confirmed by the following laboratory tests:

- Smear preparation for direct detection of acid-fast bacilli by the method of Ziehl–Neelsen;
- Histopathological examination of biopsies obtained during surgery;
- *In vitro* cultures of smears or biopsies;
- IS2404 PCR: IS2404 is an insertion sequence found in the genome of *M. ulcerans*. This insert can be used for detection of *M. ulcerans* by PCR.

In endemic areas, *in vitro* culture techniques and histopathology are most frequently not available and PCR is mostly not practicable, because of high costs and the need of well-equipped laboratories with experienced employees. Hence, the most feasible technique is direct smear examination [22,33,34].

It is important to detect Buruli ulcer in early stages of the disease, because late detection causes severe problems such as long hospitalization or extensive surgery. Unfortunately, the disease is frequently diagnosed at late stages since Buruli ulcer often remains painless without systematic symptoms [6,35]. To avoid social stigmatization, 80% of Buruli ulcer patients in West Africa consult traditional healers [32,36]. Many people do not believe in the effectiveness of surgery and fear scarring after skin-grafting. Another important aspect represents the limited geographical, financial and medical care [6,35]. Furthermore, loss of income during medical care explains late presenting of patients [32].

## 4. Challenge for the health system

Buruli ulcer was often described as a ‘disease of the poor’. Valuable proteins and nutrients are frequently missing in their food, and many poor patients do not call for medical help, until it is unavoidable [14,37]. Therefore, the only effective strategy is to encourage the population of rural, endemic areas to participate on educational programs for early detection and to raise the awareness of the existence and effectiveness of treatment. It is also important to educate people that Buruli ulcer is a result of a mycobacterial infection and not a result of sorcery [14]. Furthermore, traditional healers should be understood as link between the rural population and modern health care delivery [38]. Another model is to educate and coach volunteers to act as village health workers in affected areas [32,39–41]. The establishment of diagnostic laboratories as well as non-invasive, cheap and robust routine diagnostic tests is urgently needed in endemic areas to improve treatment chances for Buruli ulcer patients [6].

Complete surgical excision of early diagnosed lesions still remains the most effective treatment option [12]. If patients do not present in early stages of the disease, there is no alternative as wide excisions followed by skin-grafting and long hospitalization [6,42]. Concomitant physiotherapy prevents contractures [16]. To reduce the surgical excision, several medical options have been explored.

## 5. Therapy with antibiotic drugs

Until recently, surgical removal of ulcers was the first treatment of choice [6,43]. The costs for extended hospitalization can hardly be managed by local health care systems or the patients’ families [32,44,45]. Unfortunately, recurrence of the disease is very common and wide surgical excisions including healthy tissues is recommended, but oftentimes