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Drugs for treating Buruli ulcer (Mycobacterium ulcerans disease) (Review)

Yotsu RR, Richardson M, Ishii N

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[Intervention Review]

Drugs for treating Buruli ulcer (*Mycobacterium ulcerans* disease)

Rie R Yotsu[1,2], Marty Richardson[3], Norihisa Ishii[4]

[1]Department of Dermatology, National Center for Global Health and Medicine, Tokyo, Japan. [2]Department of Dermatology, National Suruga Sanatorium, Shizuoka, Japan. [3]Cochrane Infectious Diseases Group, Liverpool School of Tropical Medicine, Liverpool, UK. [4]Leprosy Research Center, National Institute of Infectious Diseases, Tokyo, Japan

Contact address: Rie R Yotsu, Department of Dermatology, National Center for Global Health and Medicine, 1-21-1 Toyama, Shinjuku-ku, Tokyo, 162-8655, Japan. yotsurie@hotmail.com.

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ABSTRACT

Background

Buruli ulcer is a necrotizing cutaneous infection caused by infection with *Mycobacterium ulcerans* bacteria that occurs mainly in tropical and subtropical regions. The infection progresses from nodules under the skin to deep ulcers, often on the upper and lower limbs or on the face. If left undiagnosed and untreated, it can lead to lifelong disfigurement and disabilities. It is often treated with drugs and surgery.

Objectives

To summarize the evidence of drug treatments for treating Buruli ulcer.

Search methods

We searched the Cochrane Infectious Diseases Group Specialized Register; the Cochrane Central Register of Controlled Trials (CENTRAL), published in the Cochrane Library; MEDLINE (PubMed); Embase (Ovid); and LILACS (Latin American and Caribbean Health Sciences Literature; BIREME). We also searched the US National Institutes of Health Ongoing Trials Register (clinicaltrials.gov) and the World Health Organization (WHO) International Clinical Trials Registry Platform (ICTRP) (www.who.int/ictrp/search/en/). All searches were run up to 19 December 2017. We also checked the reference lists of articles identified by the literature search, and contacted leading researchers in this topic area to identify any unpublished data.

Selection criteria

We included randomized controlled trials (RCTs) that compared antibiotic therapy to placebo or alternative therapy such as surgery, or that compared different antibiotic regimens. We also included prospective observational studies that evaluated different antibiotic regimens with or without surgery.

Data collection and analysis

Two review authors independently applied the inclusion criteria, extracted the data, and assessed methodological quality. We calculated the risk ratio (RR) for dichotomous data with 95% confidence intervals (CI). We assessed the certainty of the evidence using the GRADE approach.

Main results

We included a total of 18 studies: five RCTs involving a total of 319 participants, ranging from 12 participants to 151 participants, and 13 prospective observational studies, with 1665 participants. Studies evaluated various drugs usually in addition to surgery, and were carried out across eight countries in areas with high Buruli ulcer endemicity in West Africa and Australia. Only one RCT reported adequate methods to minimize bias. Regarding monotherapy, one RCT and one observational study evaluated clofazimine, and one RCT evaluated sulfamethoxazole/trimethoprim. All three studies had small sample sizes, and no treatment effect was demonstrated. The remaining studies examined combination therapy.

Rifampicin combined with streptomycin

We found one RCT and six observational studies which evaluated rifampicin combined with streptomycin for different lengths of treatment (2, 4, 8, or 12 weeks) (941 participants). The RCT did not demonstrate a difference between the drugs added to surgery compared with surgery alone for recurrence at 12 months, but was underpowered (RR 0.12, 95% CI 0.01 to 2.51; 21 participants; very low-certainty evidence).

An additional five single-arm observational studies with 828 participants using this regimen for eight weeks with surgery (given to either all participants or to a select group) reported healing rates ranging from 84.5% to 100%, assessed between six weeks and one year. Four observational studies reported healing rates for participants who received the regimen alone without surgery, reporting healing rates ranging from 48% to 95% assessed between eight weeks and one year.

Rifampicin combined with clarithromycin

Two observational studies administered combined rifampicin and clarithromycin. One study evaluated the regimen alone (no surgery) for eight weeks and reported a healing rate of 50% at 12 months (30 participants). Another study evaluated the regimen administered for various durations (as determined by the clinicians, durations unspecified) with surgery and reported a healing rate of 100% at 12 months (21 participants).

Rifampicin with streptomycin initially, changing to rifampicin with clarithromycin in consolidation phase

One RCT evaluated this regimen (four weeks in each phase) against continuing with rifampicin and streptomycin in the consolidation phase (total eight weeks). All included participants had small lesions, and healing rates were above 90% in both groups without surgery (healing rate at 12 months RR 0.94, 95% CI 0.87 to 1.03; 151 participants; low-certainty evidence). One single-arm observational study evaluating the substitution of streptomycin with clarithromycin in the consolidation phase (6 weeks, total 8 weeks) without surgery given to a select group showed a healing rate of 98% at 12 months (41 participants).

Novel combination therapy

Two large prospective studies in Australia evaluated some novel regimens. One study evaluating rifampicin combined with either ciprofloxacin, clarithromycin, or moxifloxacin without surgery reported a healing rate of 76.5% at 12 months (132 participants). Another study evaluating combinations of two to three drugs from rifampicin, ciprofloxacin, clarithromycin, ethambutol, moxifloxacin, or amikacin with surgery reported a healing rate of 100% (90 participants).

Adverse effects were reported in only three RCTs (158 participants) and eight prospective observational studies (878 participants), and were consistent with what is already known about the adverse effect profile of these drugs. Paradoxical reactions (clinical deterioration after treatment caused by enhanced immune response to *M ulcerans*) were evaluated in six prospective observational studies (822 participants), and the incidence of paradoxical reactions ranged from 1.9% to 26%.

Authors' conclusions

While the antibiotic combination treatments evaluated appear to be effective, we found insufficient evidence showing that any particular drug is more effective than another. How different sizes, lesions, and stages of the disease may contribute to healing and which kind of lesions are in need of surgery are unclear based on the included studies. Guideline development needs to consider these factors in designing practical treatment regimens. Forthcoming trials using clarithromycin with rifampicin and other trials of new regimens that also address these factors will help to identify the best regimens.

PLAIN LANGUAGE SUMMARY

Drugs for treating Buruli ulcer (*Mycobacterium ulcerans* disease)

What was the aim of this review?

The aim of this Cochrane Review was to summarize the evidence for drug treatments for Buruli ulcer.

Key messages

Antibiotics are an important component of treatment of Buruli ulcers, but there is no evidence to suggest that any particular drug is more effective than another.

What was studied in the review?

Buruli ulcer is a disease caused by mycobacterium (tuberculosis and leprosy are other types of diseases caused by mycobacterium), which results in lumps in the skin and deep ulcers, often on the arms or the face. When diagnosed late, those affected may be left with lifelong disfigurements and disabilities. The disease is most prevalent in West Africa, but it is also found in non-tropical areas including Australia and Japan. It is often treated with drugs and surgery. This review compared different drug treatments for Buruli ulcer.

What are the main results of the review?

We included 18 studies from eight countries in West Africa and Australia (1984 participants). Antibiotic combination treatments evaluated appear to be effective, but the evidence is insufficient to show that any particular drug is more effective than another.

Testing treatments in Buruli ulcer is challenging as different sizes, lesions, and stages of the disease contribute to healing rates. Surgery also plays an important role in treating Buruli ulcer, and consequently the independent effect of drugs is difficult to assess. Trials of new regimens that also address these factors will help to identify the best regimens.

How up-to-date is this review?

We searched for studies published up to 19 December 2017.

SUMMARY OF FINDINGS FOR THE MAIN COMPARISON *[Explanation]*

Rifampicin combined with streptomycin compared with surgery alone for Buruli ulcer

Patient or population: people with Buruli ulcer, non-ulcerated lesions measuring less than 10 cm in diameter, aged 15 years or older
Settings: Ghana
Intervention: rifampicin combined with streptomycin
Comparison: surgery alone

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	Number of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Surgery alone	Surgery plus rifampicin combined with streptomycin				
Recurrence, 12 months	**20 per 100**	**2.4 per 100** (< 1 to 50)	**RR 0.12** (0.01 to 2.51)	21 participants (1 trial)	⊕◯◯◯ VERY LOW[a,b] due to risk of bias and imprecision	We do not know if the treatment reduces recurrence.

*The basis for the **assumed risk** (for example, the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% CI) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
Abbreviations: CI: confidence interval; RR: risk ratio

GRADE Working Group grades of evidence
High certainty: further research is very unlikely to change our confidence in the estimate of effect.
Moderate certainty: further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low certainty: further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low certainty: we are very uncertain about the estimate.

[a] Downgraded by 1 for risk of bias: study small and not concealed.
[b] Downgraded by 2 for imprecision: very few events and wide CIs.

BACKGROUND

Buruli ulcer is a necrotizing cutaneous infection caused by infection with *Mycobacterium ulcerans* bacteria, which is categorized as a non-tuberculous mycobacterium. It is an emerging disease first described by MacCallum 1948 in six Australian patients. The disease was named after Buruli County in Uganda, where a large number of cases were reported in the 1960s (Clancey 1961; Uganda Buruli Group 1970). Since then, the number of Buruli ulcer cases has gradually increased (Yotsu 2015). In spite of this, the disease is still poorly understood, especially its transmission mode. Several studies have demonstrated that the infection is linked to aquatic environments (Lunn 1965; Bradley 1971; Marsollier 2002; Eddyani 2004; Johnson 2005b). However, the natural reservoir and mode of transmission of the infection remain a mystery and may differ between endemic foci worldwide (Merritt 2010).

Currently, over 33 countries worldwide report cases of Buruli ulcer, mainly in people living in tropical and subtropical regions (WHO 2013). About 2000 to 5000 new cases are reported annually, mostly in countries in West and Central Africa (WHO 2013). Most people who are infected in these countries are children aged under 15 years, living in remote rural areas with limited access to health facilities (Marston 1995; Asiedu 1998; Phanzu 2006; Wansbrough-Jones 2006). Other important foci include Australia (Boyd 2012; Tai 2018), French Guiana (Couppié 2015), Papua New Guinea (Igo 1988; Joseph 2003), and more recently, Japan (Yotsu 2012). In addition, a number of cases have been reported in international travellers from non-endemic areas, including North America and European countries (van Oye 1950; Farber 1967; Bär 1998; Semret 1999; Faber 2000; Evans 2003; Ezzedine 2009). Nevertheless, awareness and knowledge of the disease among health practitioners and the community are still lacking, hence the possibility of hidden unreported cases (WHO 2013). In endemic countries, poor health infrastructure and geographical challenges also contribute to the underreporting of cases (WHO 2013). If left undiagnosed and untreated, the disease can lead to lifelong disfigurement and disabilities, which impact greatly on the lives of those affected, especially in resource-poor conditions where most of these people reside.

Description of the condition

The subcutaneous tissue is the primary site of infection by *M ulcerans* (van der Werf 1999). The bacteria produce mycolactone, an immunomodulatory macrolide toxin, which is the main pathogenic factor of the disease. This toxin induces tissue necrosis, particularly in subcutaneous fat (van der Werf 2003). Initially, the disease presents as a nodule, papule, plaque (firm, painless, and raised lesion, which is larger than a papule), or oedema, which when left alone eventually breaks open the skin and forms an ulcer. A typical ulcer usually has necrotic slough, undermined edges, and is often painless (unless complicated with a secondary infection) (van der Werf 1999). *M ulcerans* infection often affects the upper and lower limbs and the face, as these are exposed body areas. It can progress sideways to become a larger lesion involving the joints, as well as deeper into the tissue and cause osteomyelitis in some cases. However, it is rare for the infection to disseminate systemically and cause death (Sizaire 2006). If death occurs, it is usually related to sepsis from a secondary infection or tetanus (van der Werf 1999).

The World Health Organization (WHO) has classified Burui ulcer lesions into three groups according to important clinical features and size, with implications for their management (WHO 2012). Category I is a small, early lesion less than 5 cm in diameter; category II is a lesion of 5 to 15 cm in diameter; and category III is a lesion more than 15 cm in diameter, multiple lesions, or lesion(s) at a critical site (eye, breast, genitalia) and osteomyelitis (WHO 2012). Some people experience spontaneous healing during the course of the disease, but the mechanism for this is unclear (Johnson 2005a; Gordon 2011). In severe cases, lifelong sequelae may develop. Vincent 2014a reported that among their 1043 laboratory-confirmed cases of Buruli ulcers in Benin, 229 people (22%) developed permanent functional impairment one year after their treatment.

The association between Buruli ulcer and HIV/AIDS is not yet clear; there have been some reports on the possible increased rate of infection and severity in those with HIV/AIDS (Vincent 2014b; Tuffour 2015).

Diagnosis

Buruli ulcer possesses characteristic clinical features, and hence clinical diagnosis is possible to a certain extent in endemic areas. However, for definitive diagnosis, laboratory microbiological methods are required, including Ziehl-Neelsen (ZN) staining for detecting acid-fast bacilli (AFB), in vitro culture, polymerase chain reaction (PCR) assay targeting genomic region IS2404, and histopathology. Findings from at least one of these laboratory microbiological methods should be suggestive of Buruli ulcer to confirm diagnosis (WHO 2014). Samples can be obtained by fine-needle aspiration from a non-ulcerative lesion, and purulent discharge fluid or swab from the undermined wound edge of an ulcerative lesion. Skin biopsy is a reliable sample source, but this can only be performed with adequate skills, tools, and hygienic environment, which may be limited in places where Buruli ulcer is endemic. The WHO is currently promoting PCR confirmation for at least 70% of all reported cases of Buruli ulcer (WHO 2014).

Description of the intervention

Since the first description of the disease in 1948, the standard treatment for Buruli ulcers was extensive surgical debridement of affected skin and surrounding tissue, with or without subsequent

skin grafting (Darie 1994; van der Werf 2003). However, surgical treatment alone was insufficient to eradicate all the *M ulcerans* bacteria, and recurrence was common. Although the recurrence rate varied between studies, it was reported to be from 6% to 32% (Amofah 1998; Kanga 2003; Debacker 2005; Kibadi 2006; O'Brien 2013a). Moreover, surgery is available only to a small fraction of the population in the most affected areas of low- and middle-income countries due to limited hospital capacities, and difficulties relating to accessibility and cost (WHO 2004). Lesion site is another challenge. If the ulcer involves the face, joints, or other important body parts, which is not a rare occurrence in people with Buruli ulcer, surgical excision may cause disfiguring or disabling consequences (Sizaire 2006). For these reasons, there has been a continuous exploration for other medical approaches that can effectively cure Buruli ulcer, including topical treatments using nitrogen oxide (Phillips 2004a; Phillips 2004b), phenytoin powder (Klutse 2003), local heat treatment (Meyers 1974; Krieg 1979; Junghanss 2009; Vogel 2016), hyperbaric oxygen therapy (Krieg 1975; Krieg 1979), and antibiotic treatments (WHO 2004; WHO 2012; WHO 2017).

Several trials of different antibiotic treatments have been conducted, including clofazimine and sulfamethoxazole/trimethoprim (Revill 1973; Fehr 1994), but results of these monotherapies were disappointing. Rifampicin, when used alone, caused the development of a rifampicin-resistant *M ulcerans* strain in a mice model, suggesting that it should never be used as monotherapy in people, as in people with tuberculosis (TB) or leprosy (Marsollier 2003). In 2004, based on in vitro findings and pilot clinical studies, the WHO introduced a combination of rifampicin (10 mg/kg orally once daily) and streptomycin (15 mg/kg intramuscularly once daily) for eight weeks (critical base drugs in TB) as a first-line therapy for people with Buruli ulcer (WHO 2004), which has greatly simplified the treatment and delivery of care for those affected. Nevertheless, surgical treatment adjunctive to antibiotics still plays an important role in Buruli ulcer management, especially for people with severe, large ulcers. The WHO recommends surgical intervention for category III cases and some category II cases, following careful assessment of the efficacy of the antibiotic treatment. In Buruli ulcer, surgical debridement is performed extensively with a wide margin, as mycolactone exists in the subcutaneous fat tissue beyond the wound edges.

Despite antibiotic treatment being effective to an extent, some concerns remain with the current recommended regimen. Streptomycin requires intramuscular injection, which is invasive, therefore patient acceptance and adherence are affected. It is also operationally demanding and of limited availability to people living in remote areas where Buruli ulcer is most endemic, especially rural Africa. Additionally, in these areas, administration of drugs by injection carries the risk of HIV transmission. Potential adverse effects from streptomycin, including ototoxicity and nephrotoxicity, are another concern. There is also concern about encouraging the development of multidrug-resistant TB, as both rifampicin and streptomycin are also effective antituberculosis drugs. Active TB would need to be confidently ruled out before treatment, and considering that this judgement may not always be completely accurate, there may be substantial consequences for the future of TB treatment. The search for a fully orally administered treatment regimen to replace rifampicin and streptomycin combination for the treatment of Buruli ulcer is thus ongoing. Several options have already been explored as replacements for the curative rifampicin and streptomycin combination, including: rifampicin and dapsone (Espey 2002), rifampicin and clarithromycin (BURULICO Study 2010; Chauty 2011; Phillips 2014a; Friedman 2016), rifampicin and ciprofloxacin (O'Brien 2012; Friedman 2016), and rifampicin, levofloxacin, and clarithromycin (Sugawara 2015).

To date, evaluating the efficacy of treatments for Buruli ulcer has been challenging for several clinical and biological reasons. Firstly, there have been cases in which deterioration was observed during the course of treatment, which are now defined as paradoxical reactions. This phenomenon is now understood to be the result of antibiotic suppression of mycolactone synthesis, leading to the reversal of host immune response to*M ulcerans* (Nienhuis 2012). Paradoxical reactions may occur at the same site as the initial lesion, or at other sites. When it is at the same site, it is especially difficult to differentiate paradoxical reactions from recurrences; this identification largely influences the clinical decision. The WHO defines recurrences as new and culture-confirmed lesions occurring more than three months after completion of antibiotic treatment (WHO 2012). However, the two conditions cannot be fully differentiated based on this definition alone. Since paradoxical reactions have only recently been documented, some past data on recurrences may have mistakenly included paradoxical reactions. Secondly, microbiological cure and clinical cure are not always the same. In other words, even though *M ulcerans* was successfully eliminated from the lesion site with antibiotic treatment (microbiological cure), this does not correspond to clinical cure if the patient has already manifested an ulcer. Moreover, in such ulcerated cases, methods used in wound care would also modify the healing process; this is another challenge in correctly evaluating antimicrobial treatment efficacy in people with Buruli ulcer. Selection of wound care methods is often dependent upon daily practice and resource availability. Velding 2014 documented that there was a wide diversity in local wound care methods practiced by health practitioners/healthcare givers in Ghana and Benin. Due to these atypical clinical features and medical practices related to the disease, it has been difficult to develop a clear case definition for cure. Many studies evaluating treatment efficacy in Buruli ulcer disease have used complete epithelialization, Chauty 2007; Sugawara 2015, or reduction in wound size, Etuaful 2005; BURULICO Study 2010; Sugawara 2015, as their definition of cure (clinical cure), while a few studies have also used microbiological cure as their case definition of cure, employing laboratory methods (Etuaful 2005; Sarfo 2010).

How the intervention might work

As Buruli ulcer is a mycobacterial disease and with growing experience in its management, antibiotic drugs are now an essential part of its treatment (WHO 2012; Yotsu 2015). After the introduction of antibiotic drugs for the treatment of Buruli ulcer by the WHO in 2004, recurrence rates reportedly decreased substantially to 0% to 2%, and the need for surgical intervention has diminished (Chauty 2007; BURULICO Study 2010; Sarfo 2010). With this simplified treatment and delivery of care, the quality of life of patients has increased not only during treatment, but also after treatment as use of antibiotic drugs has played a role in decreasing the number of those affected by the disease who are left with disabilities and disfigurements (Klis 2014c). In West Africa, where over 40% of those affected are children under 15 years of age, better treatment further provides better opportunity for education, and thus a better future (Agbenorku 2011; WHO 2012). The use of antibiotic drugs has also decreased the socioeconomic impact on families, as the cost of treatment of surgeries and hospitalization is far beyond the means of those most severely affected (Asiedu 1998; Grietens 2008; Agbenorku 2011).

Why it is important to do this review

No systematic review of the literature on Buruli ulcer has previously been performed. A review of the efficacy of daily administration of rifampicin and streptomycin in the treatment of early-stage Buruli ulcer including data from 2005 to 2012 was published in 2013 (Vouking 2013). In that review, evidence of diagnostic accuracy and ascertainment of cure was not clear. Also, the review did not include treatment modalities other than rifampicin and streptomycin. In this Cochrane Review, we aimed to assess the effects of antibiotic treatment with or without surgical intervention (debridement, skin grafting, etc.) for people with Buruli ulcer. As the search for more efficacious and/or convenient treatment modalities continues, it was an appropriate time to evaluate and summarize the evidence on current treatment options.

OBJECTIVES

To summarize the evidence of drug treatments for treating Buruli ulcer.

METHODS

Criteria for considering studies for this review

Types of studies

Randomized controlled clinical trials (RCTs) and prospective observational studies.

Types of participants

We included participants diagnosed as having Buruli ulcer due to the presence of a suggestive lesion and any one of the following:

- a culture of *M ulcerans* from the lesion;
- a positive IS2404 dry-reagent-based PCR from a swab or biopsy of the lesion;
- histopathological finding indicative of *M ulcerans* infection (for example, necrotic granuloma, presence of AFB), irrespective of age.

Types of interventions

We included studies that compared:

- antibiotic therapy to placebo or alternative therapy such as surgery;
- different antibiotic regimens.

We also included prospective observational studies that evaluated different antibiotic regimens with or without surgery.

Types of outcome measures

Primary outcomes

- Cure: healing of skin lesions without recurrence at 12 months or longer.
- Probable cure: healing of skin lesions with follow-up to 12 months.
- Possible cure: healing of skin lesions at follow-up.

Secondary outcomes

- Surgery.
- Healing time needed for wound closure.
- Reduction in ulcer size.
- Recurrence of skin lesion(s) after healing.
- Adverse effects.
- Paradoxical reactions.

Search methods for identification of studies

We attempted to identify all potential studies regardless of language or publication status (published, unpublished, in press, and in progress).

Electronic searches

We searched the following databases using the search terms and strategy described in Appendix 1: the Cochrane Infectious Diseases Group Specialized Register; the Cochrane Central Register of Controlled Trials (CENTRAL), published in the Cochrane Library (Issue 11, 2017); MEDLINE (PubMed; from 1966); Embase (Ovid; from 1947); and LILACS (Latin American and Caribbean Health Sciences Literature; BIREME) (from 1982). All searches were conducted on 19 December 2017. We also searched the US National Institutes of Health Ongoing Trials Register ClinicalTrials.gov (clinicaltrials.gov) and the World Health Organization (WHO) International Clinical Trials Registry Platform (ICTRP) (www.who.int/ictrp/search/en/) up to 19 December 2017 using "Buruli ulcer*" as a search term.

Searching other resources

We reviewed the reference lists of all included studies. We also contacted leading researchers in this topic area to identify any unpublished data.

Data collection and analysis

Selection of studies

Vittoria Lutje, the Cochrane Infectious Diseases Group (CIDG) Information Specialist, searched the literature and retrieved studies using the search strategy outlined in Appendix 1. In the initial stage of selection, two review authors (Rie Roselyne Yotsu (RRY) and Marty Richardson (MR)) independently screened the abstracts of studies retrieved by the search to identify those that met the inclusion criteria. We retrieved the full-text articles of published or unpublished potentially relevant study reports for further assessment. Rie Roselyne Yotsu or Marty Richardson contacted the study authors for further details regarding study methodology if eligibility was unclear. A third review author (Norihisa Ishii (NI)) was consulted when there was a difference of opinion between RRY and MR. If there was still disagreement between the review authors, we consulted one of the CIDG Co-ordinating Editors to reach a consensus. We examined study reports to ensure that we included multiple publications from the same study only once.

Data extraction and management

Two review authors (RRY and MR) extracted and summarized data from the included studies on standardized data extraction forms. Any differences of opinion were resolved through discussion. If important data were missing from the included studies, we contacted the study authors for further information.

We extracted the number of participants randomized and the number of participants followed up in each treatment arm, with a list of each study's inclusion and exclusion criteria, a description of the intervention(s), and primary and secondary outcome measures. The data extraction form also included baseline characteristics of participants in the control group such as age, sex, stage of lesions, ulcer size, WHO category, diagnostic results, healing time, side effects, outcome, post-treatment surgery, and recurrence. Rie Roselyne Yotsu entered the data into Review Manager 5 (RevMan 2014).

For dichotomous outcomes, we extracted the number of participants experiencing the event and the number of participants in each treatment group. For continuous outcomes, we extracted arithmetic means, standard deviations, and the numbers of participants for each treatment group.

Assessment of risk of bias in included studies

All review authors (RRY, MR, and NI) independently assessed the risk of bias for each included study. We assessed RCTs using the Cochrane 'Risk of bias' assessment tool with seven domains of bias including: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other potential sources of bias (Higgins 2011). We assessed prospective observational studies in accordance with methods adopted from 'A Cochrane Risk of Bias Assessment Tool: for Non-Randomized Studies of Interventions' (ACROBAT-NRSI) (Sterne 2014). We assessed five domains of bias including: selection of participants into the study, measurement of outcomes, incomplete outcome data, selective reporting, and other potential sources of bias.

We assigned a judgement of either 'high', 'low', or 'unclear' risk of bias for each component. We chose 'unclear' either when the available information was inadequate to judge or when it was neither 'high' nor 'low'. Any discrepancies regarding 'Risk of bias' analysis results were resolved through discussion. We consulted one of the CIDG Co-ordinating Editors if necessary. We presented the findings in a 'Risk of bias' table, and produced figures to summarize the risk of bias across included studies. For domains that did not pertain to the study design, we assigned 'unclear risk of bias' for RCTs and 'low risk of bias' for prospective observational studies so that all studies could be handled in a single 'Risk of bias' graph and summary figure. We also labelled the study name and the domains with the study design in order to enable differentiation between the two study designs.

We further assessed the certainty of the evidence using the GRADE approach for any RCTs for which we could apply this method (Juni 2001). We used GRADEpro GDT software to construct a 'Summary of findings' table (GRADEpro GDT 2015).

Measures of treatment effect

For RCTs using dichotomous outcomes, we presented the effect of treatment within studies as the risk ratio (RR) with corresponding 95% confidence interval (CI).

Unit of analysis issues

Had we identified studies for inclusion that had multiple intervention arms, we would have included data from these studies by either combining treatment arms, or by splitting the control group so that participants would only be included in the meta-analysis once.

Dealing with missing data

In the case of missing data, we attempted to contact the study authors to request the missing information. If the study authors did not collect or assess the needed data as part of their study, or if we received no response, we analysed the available data only using a complete-case analysis.

Assessment of heterogeneity

Had we performed meta-analyses in this review, we would have inspected forest plots visually to assess whether statistical heterogeneity was present. We would have deemed CIs that did not overlap as indicating statistical heterogeneity.

Assessment of reporting biases

We planned to assess reporting bias by using funnel plots, however we did not create these as we did not perform any meta-analyses in this review.

Data synthesis

We compared studies in terms of combination of antibiotics and duration, whether adjunctive surgery was performed or not, and lesion size/types in order to determine whether it was possible, and appropriate, to perform meta-analyses. We consequently decided that it was not possible to perform meta-analyses due to the small number of studies with the same intervention, different inclusion criteria (for example, some studies only included small lesions while others included large lesions; some studies only included ulcerated lesions while others included non-ulcerated lesions), and different follow-up/assessment time points. We presented the key characteristics of included studies alongside outcome data in tables, and discussed the results of the included studies narratively. We will refer to the methods described in the protocol should we need to conduct analyses in future updates.

Subgroup analysis and investigation of heterogeneity

Had we detected substantial heterogeneity in meta-analyses, we would have explored the possible causes of the heterogeneity by performing subgroup analyses. Subgroups for investigation included lesion sizes, clinical lesions (papule, nodule, plaque, oedema, and ulcer), and surgical intervention.

Sensitivity analysis

We did not perform sensitivity analyses as we did not perform any meta-analyses in this review.

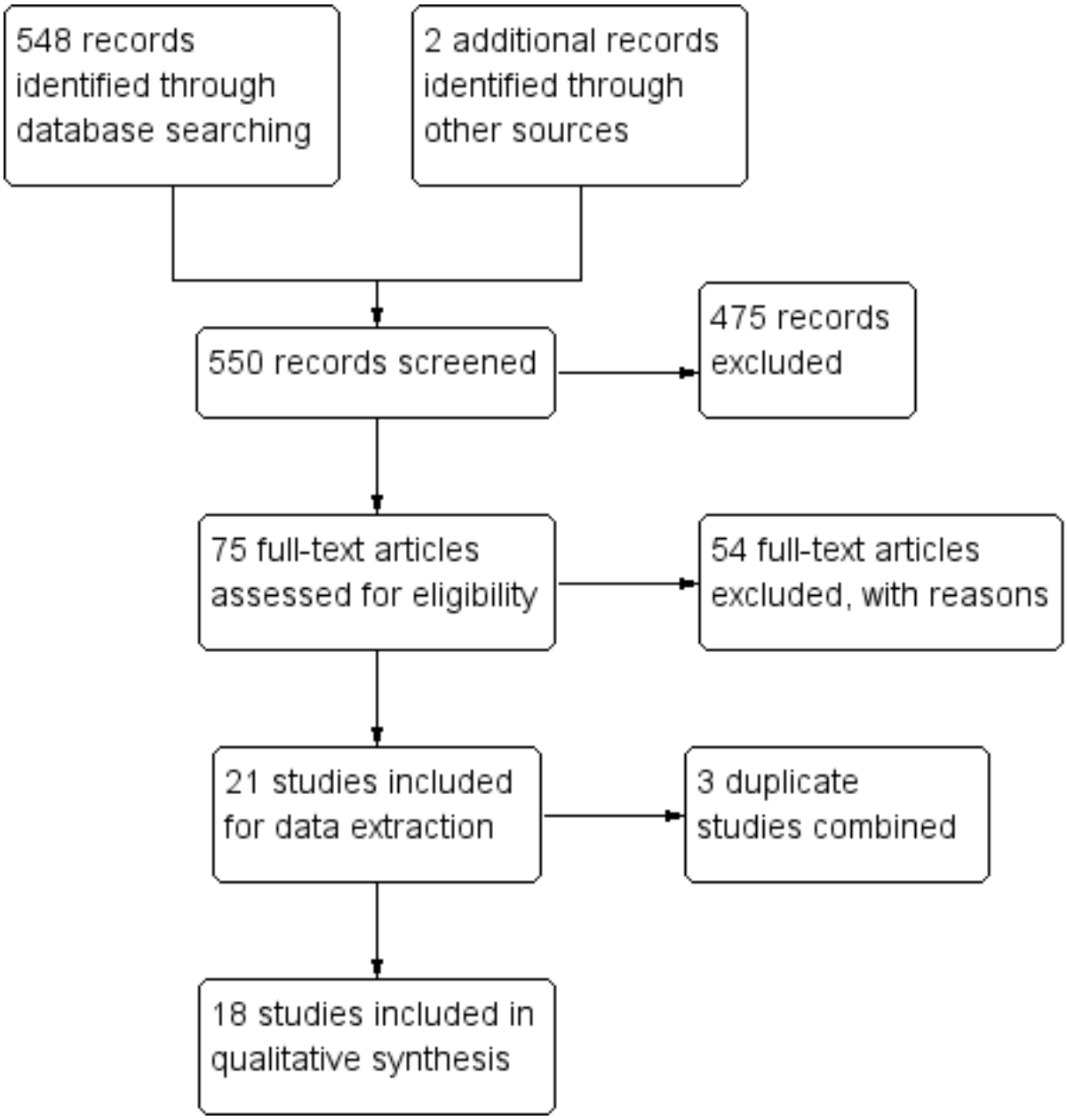
RESULTS

Description of studies

Results of the search

We conducted literature searches up to 19 December 2017 and identified 548 titles (Figure 1). While searching for additional information on Arens 2015, we identified one study through its conference proceeding (Beissner 2015), and one study through ongoing trials (Barogui 2016). Two review authors (RRY and MR) closely examined 75 full-text articles. We contacted the technical team at the WHO for possible unpublished studies; there were none other than those we identified. We identified two ongoing trials on US National Institutes of Health Ongoing Trials Register ClinicalTrials.gov (NCT01432925; NCT01659437).

Figure 1. Study flow diagram.



We identified six RCTs and 15 prospective observational studies that met the inclusion criteria. Two review authors (RRY and MR) independently extracted data for these studies.

Included studies

Study design

Six articles reported a total of five RCTs. The BURULICO study was reported in two different articles with different outcomes (Nienhuis 2010; Klis 2014; see BURULICO Study 2010).

A total of 15 articles reported prospective observational studies. Five articles were from the same Australian group using the data of Buruli ulcer patients from their registry that they had started collecting in January 1998 (O'Brien 2007; O'Brien 2012; Friedman 2013; O'Brien 2013b; Friedman 2016), and evaluated a number of different combinations of antibiotics. We identified two sets of articles reporting data for the same groups of participants at different time points (Friedman 2013 and Friedman 2016; O'Brien 2007 and O'Brien 2012), therefore we extracted data from only the more recent papers (O'Brien 2012; Friedman 2016). Some participants in O'Brien 2012, O'Brien 2013b, and Friedman 2016 may be included in more than one study, as they were from the same registry. Barogui 2016 combined participants of the BURULICO Study 2010 and the ongoing NCT01432925 trial, therefore there is overlap of participants. However, Barogui 2016 measured an outcome (paradoxical reactions) that was not an outcome measure of the original RCTs. We counted this study as an independent, prospective observational study.

We henceforth describe results of the qualitative synthesis of five RCTs and 13 prospective observational studies.

Interventions and comparisons

Randomized controlled trials

The included RCTs evaluated the following.

- Monotherapy in comparison to placebo, with surgery when indicated: clofazimine in one trial (Revill 1973), and sulfamethoxazole/trimethoprim in a second trial (Fehr 1994).
- Combination therapy:
  - rifampicin + streptomycin before surgery with different lengths of treatment (varying from two to 12 weeks), in comparison to surgery alone (Etuaful 2005);
  - rifampicin + streptomycin for four weeks followed by rifampicin + clarithromycin for four weeks in comparison to rifampicin + streptomycin for eight weeks, with surgery when indicated (BURULICO Study 2010);
  - rifampicin + dapsone for eight weeks in comparison to no treatment, with no surgery in either arm (Espey 2002).

Prospective observational studies

Two studies evaluated different treatment regimens in multiple treatment arms (O'Brien 2012; Friedman 2016). All of the other prospective observational studies were single-arm studies. Prospective observational studies evaluated the following.

- Monotherapy with clofazimine for one to four weeks before surgery (Lunn 1964).
- Combination therapy with rifampicin + streptomycin for:
  - 12 weeks with surgery at week 4 (Kibadi 2010);
  - eight weeks with surgery when indicated (Chauty 2007; Sarfo 2010; Adu 2013; Beissner 2015);
  - eight weeks with surgery (Agbenorku 2011).
- Combination therapy with rifampicin + clarithromycin:
  - rifampicin + clarithromycin for eight weeks, with surgery when indicated (Chauty 2011);
  - rifampicin + streptomycin for two weeks followed by rifampicin + clarithromycin for six weeks, with surgery when indicated (Phillips 2014a).
- Other combination therapy:
  - rifampicin + either ciprofloxacin, clarithromycin, or moxifloxacin, with no surgery or with limited debridement (Friedman 2016);
  - rifampicin + ciprofloxacin, rifampicin + clarithromycin, rifampicin + clarithromycin + ethambutol, ciprofloxacin + clarithromycin, rifampicin + moxifloxacin, clarithromycin + ethambutol, rifampicin + ethambutol + amikacin, or clarithromycin only, with surgery in all cases, in comparison to surgery alone (O'Brien 2012);
  - single or combination administration of rifampicin, ciprofloxacin, clarithromycin, ethambutol, amikacin, and/or moxifloxacin, with surgery when indicated (O'Brien 2013b);
  - either rifampicin + streptomycin for eight weeks or rifampicin + streptomycin for four weeks followed by rifampicin + clarithromycin for four weeks, with surgery when indicated (Barogui 2016).

Location and participants

All studies were conducted in areas with high Buruli ulcer endemicity: of the RCTs, three were conducted in Ghana and one in Côte d'Ivoire and in Uganda; of the prospective observational studies, four were conducted in Ghana, three in Australia, two in Benin, one in Uganda, one in Democratic Republic of Congo, and one in Togo. Barogui 2016 was a joint study between Ghana and Benin.

Some studies set inclusion criteria for age and lesion type or size given in diameter. Of the RCTs, the BURULICO Study 2010 recruited participants over five years with lesion size less than 10 cm; Etuaful 2005 recruited participants over 15 years with lesion size less than 10 cm; and Espey 2002 recruited participants over four years with ulcers. Of the prospective observational studies, Chauty 2011 recruited participants over five years with lesion size less than 10 cm; Phillips 2014a recruited participants over five years with lesion size less than 15 cm; Kibadi 2010 recruited participants between three and 75 years with lesion size larger than 10 cm; and the NCT01432925 trial (a part of Barogui 2016) recruited participants over three years of age. All other included studies recruited all age groups and lesion sizes.

Three RCTs, Fehr 1994; Etuaful 2005; BURULICO Study 2010, and 10 prospective observational studies, Sarfo 2010; Agbenorku 2011; Chauty 2011; O'Brien 2012; Adu 2013; O'Brien 2013b; Phillips 2014a; Beissner 2015; Barogui 2016; Friedman 2016, had laboratory confirmation as part of their inclusion criteria. The remaining included studies did not have laboratory confirmation as an inclusion criterion.

Outcomes and length of follow-up

Outcomes in the RCTs varied. One trial measured "cure" (BURULICO Study 2010), and one trial measured "possible cure" (Revill 1973). Both trials also measured healing time (Revill 1973; BURULICO Study 2010). Otherwise, change in ulcer size was investigated in three trials (Fehr 1994; Espey 2002; Etuaful 2005), recurrence in three trials (Revill 1973; Etuaful 2005; BURULICO Study 2010), and adverse effects in three trials (Espey 2002; Etuaful 2005; BURULICO Study 2010).

Of the prospective observational studies, seven studies measured "cure" (Phillips 2004; Kibadi 2010; Sarfo 2010; Agbenorku 2011; Chauty 2011; O'Brien 2012; Friedman 2016); one study measured "probable cure" (Chauty 2007); and three studies measured "possible cure" (Lunn 1964; Adu 2013; Beissner 2015). Healing time was investigated in five studies (Sarfo 2010; Chauty 2011; Phillips 2014a; Beissner 2015; Friedman 2016), change in ulcer size in one (Sarfo 2010), recurrence in eight (Chauty 2007; Kibadi 2010; Sarfo 2010; Agbenorku 2011; Chauty 2011; O'Brien 2012; Phillips 2014a; Beissner 2015), adverse effects in eight (Lunn 1964; Chauty 2007; Sarfo 2010; Agbenorku 2011; Chauty 2011; O'Brien 2012; Phillips 2014a; Friedman 2016), and paradoxical reactions in six studies (Sarfo 2010; O'Brien 2012; O'Brien 2013b; Phillips 2014a; Barogui 2016; Friedman 2016).

Follow-up period varied in the RCTs. Etuaful 2005 followed up participants until one year after completion of treatment. In the BURULICO Study 2010, Nienhuis and colleagues first followed up participants until one year, and then Klis and colleagues revisited participants again during four to six years after treatment. Two trials did not specify their follow-up time (Fehr 1994; Espey 2002). In the earlier study by Revill 1973, their follow-up period ranged from 17 to 40 months, with a median of 32 months. Follow-up in the prospective observational studies was one year in six studies (Chauty 2007; Sarfo 2010; O'Brien 2012; O'Brien 2013b; Phillips 2014a; Friedman 2016). Otherwise, it was seven months in Barogui 2016, 1.5 years in Chauty 2011, two years in Agbenorku 2011 and Kibadi 2010, and not specified in Lunn 1964, Adu 2013, and Beissner 2015.

Excluded studies

We excluded 475 studies after title and abstract screening. We assessed 75 full-text articles for eligibility, of which we excluded 37 on the basis of their study design (retrospective observational studies, cross-sectional surveys, case series, or qualitative studies), eight because they were either reviews or commentaries, five because they were conference proceedings, and four because they were duplicates.

Risk of bias in included studies

We have summarized the risk of bias in included studies in Figure 2 and Figure 3.

Figure 2. 'Risk of bias' graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.

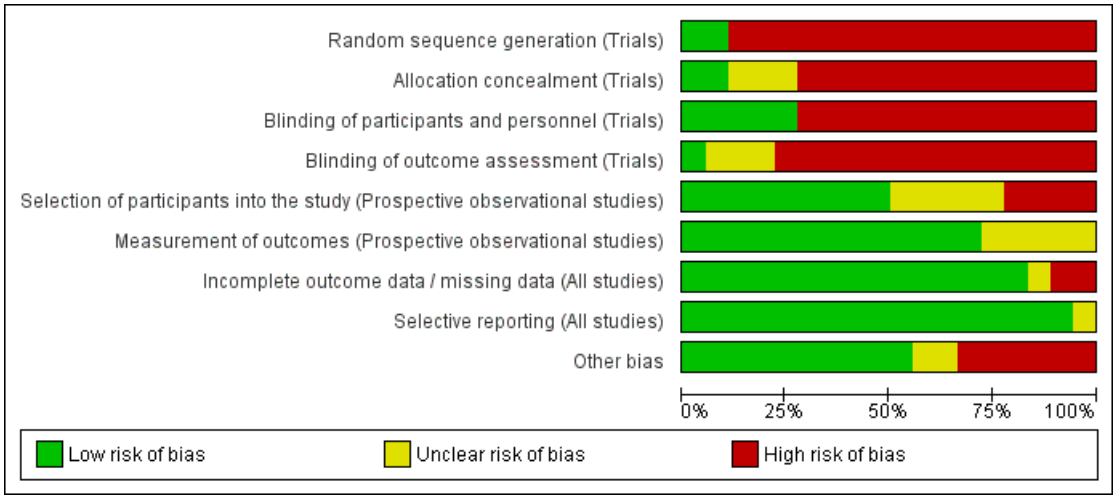
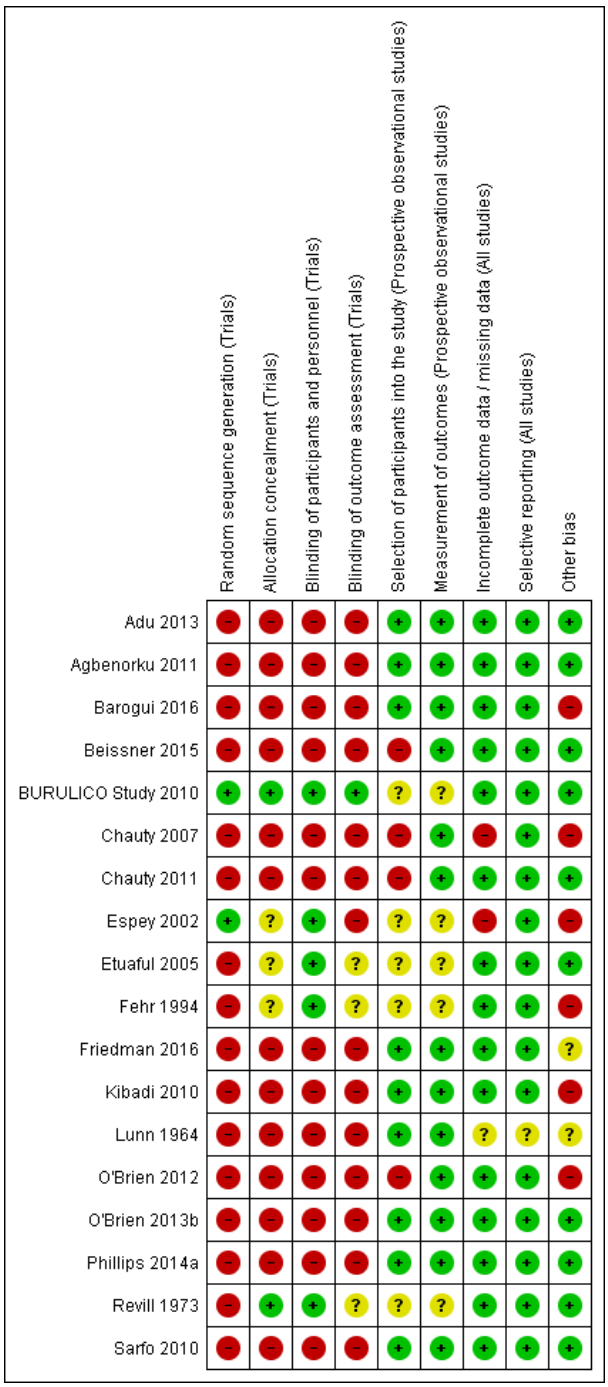


Figure 3. 'Risk of bias' summary: review authors' judgements about each risk of bias item for each included study.



Randomized controlled trials

Of the five included RCTs, only BURULICO Study 2010 used adequate methods. Otherwise, either methods were either inadequate or details were poorly reported in the remaining studies.

Prospective observational studies

Of the 13 prospective observational studies, we rated seven recent studies as at low risk of bias (Sarfo 2010; Agbenorku 2011; Adu 2013; O'Brien 2013b; Phillips 2014a; Barogui 2016; Friedman 2016). The older studies were associated with a higher risk of bias (Lunn 1964; Chauty 2007).

Allocation

Of the five RCTs, two were blinded and were rated as at low risk of bias (Revill 1973; BURULICO Study 2010). Otherwise, no information, Espey 2002; Etuaful 2005, or no clear statement, Fehr 1994, was provided, and these studies were rated as at unclear risk of bias.

Blinding

Of the five RCTs, two were blinded and were rated as at low risk of bias (Revill 1973; Fehr 1994). Otherwise, the RCTs were open-label, BURULICO Study 2010, or no clear statement was provided, Espey 2002; Etuaful 2005, but were rated as at low risk of bias as the outcome was unlikely to be influenced by lack of blinding.

Incomplete outcome data

Of the five RCTs, we rated one as at high risk of bias, as 10 out of 30 participants (33%) were lost to follow-up (Espey 2002). The proportion of missing data was relatively large in one RCT (6/18 participants, 33%) (Fehr 1994), however reasons for exclusions/missing data were relatively well balanced or unlikely to be related to true outcome, and the RCT was rated as at low risk of bias. Otherwise, no participants, Etuaful 2005, or a minimal number of participants, Revill 1973; BURULICO Study 2010, were lost to follow-up, and we judged these RCTs as at low risk of bias.

Of the 13 prospective observational studies, we rated two studies as at high risk of bias: the assessment time point was unclear in Lunn 1964, and 17 participants were lost to follow-up during the study period but were included in the final analysis in Chauty 2007. Otherwise, either no participants, Kibadi 2010; Agbenorku 2011; Chauty 2011; O'Brien 2012; Adu 2013; O'Brien 2013b; Beissner 2015; Barogui 2016; Friedman 2016, or a minimal number of participants, Sarfo 2010; Phillips 2014a, were lost to follow-up, and we considered these studies as at low risk of bias.

Selective reporting

Of the five included RCTs, we rated one as at unclear risk of bias as there were no predefined outcomes (Lunn 1964). All of the other RCTs reported all expected outcomes, and we rated these as at low risk of bias.

All 13 prospective observational studies reported all expected outcomes and were rated as at low risk of bias.

Other potential sources of bias

Five studies either did not have laboratory confirmation as their inclusion criteria or only performed laboratory exams in a portion of their participants, therefore non-Buruli ulcer cases may be included in their study results (Lunn 1964; Revill 1973; Espey 2002; Chauty 2007; Kibadi 2010). The standard treatment for Buruli ulcer has transitioned from surgery to drugs plus surgery as adjunctive treatment after the recommendation of drug treatment by the WHO in 2004 (WHO 2014), and this may have created some bias.

Potential comorbidities such as osteomyelitis, HIV/AIDS, diabetes mellitus, cancer, and use of immunosuppressant drugs may have affected some results, especially on severity and healing rate and time. Two studies reported on comorbidities of their study participants: 9.5% in Friedman 2016 and 16.3% in O'Brien 2012; there may be an overlap of participants in these two studies.

Effects of interventions

See: **Summary of findings for the main comparison** Rifampicin combined with streptomycin compared with surgery alone for Buruli ulcer; **Summary of findings 2** Rifampicin with clarithromycin compared with rifampicin with streptomycin in the consolidation phase for Buruli ulcer

We first assess the effects of a variety of treatments on healing and recurrence, stratified by monotherapy and combination therapy. We then summarise adverse effects and paradoxical reactions across all comparisons.

Healing and recurrence

Monotherapy

See Table 1.

One RCT and one prospective observational study evaluated the efficacy of clofazimine, and one RCT evaluated the efficacy of sulfamethoxazole/trimethoprim. All three studies had small sample sizes, and no treatment effects were demonstrated.

Clofazimine

Revill 1973 compared clofazimine to placebo, with similar recurrence in the two arms (clofazimine 8/51 (15.7%); placebo 10/54 (18.5%); difference 2.8%, 95% confidence interval (CI) not given). The authors examined a subgroup of participants with non-ulcerated lesions who were withheld from immediate surgery: the number that healed was slightly higher with clofazimine, but the difference was small, and this was a post hoc subgroup analysis (clofazimine, 5/13 (38%); placebo, 6/21 (29%)). The median healing time was measured in this same subgroup also those with a lesion less than 5 cm in diameter (clofazimine, 8 participants; placebo, 17 participants) and was 21 weeks and 14 weeks, respectively.

One prospective observational study, Lunn 1964, examined the effects of clofazimine with surgery in 10 participants with ulcers. Six participants (60%) achieved complete healing in 3 to 12 weeks. The remaining four participants were still under treatment for their ulcers at the time of reporting.

Sulfamethoxazole/trimethoprim

Fehr 1994 compared sulfamethoxazole/trimethoprim to placebo in 12 participants with ulcers. The mean ulcer size in the sulfamethoxazole/trimethoprim group at baseline was 73.8 cm^2 (9 to 247) and in the placebo group was 38.7 cm^2 (15 to 80). The authors reported that sulfamethoxazole/trimethoprim reduced ulcer size by an average of 10.9%, while an average increase of 24.5% was observed in the placebo group (P = 0.15). The percentage ulcer area covered by granulation tissue at study end was 92% in the sulfamethoxazole/trimethoprim group and 57% in the placebo group (P = 0.17).

Combination therapy

Rifampicin combined with streptomycin

See Table 2.

One RCT and six prospective observational studies investigated the efficacy of rifampicin and streptomycin. Five prospective observational studies evaluated this regimen administered for 8 weeks (828 participants) with surgery given to either all participants or a select group. Four studies reported healing rates for all participants, regardless of whether they had received surgery or not (84.5% to 100%, assessed at various time points). Four studies reported healing rates for participants who received combination therapy alone (48% to 95%, assessed at various time points).

One RCT, Etuaful 2005, examined 21 participants with non-ulcerative lesions to test the efficacy of rifampicin and streptomycin. They divided the participants into 5 groups: 4 groups were given rifampicin and streptomycin for 2, 4, 8, and 12 weeks before surgery respectively, and one group received only surgery. No recurrence was observed in participants in any group receiving combination therapy at 12 months, compared with one case of recurrence in a participant who received only surgery. No difference in recurrence was observed between these two groups (risk ratio (RR) 0.12, 95% CI 0.01 to 2.51; Figure 4; Analysis 1.1). Reduction in lesion surface area in participants who received rifampicin and streptomycin was the highest (52%) in the group that underwent four weeks of the regimen before surgery.

Figure 4. Forest plot of comparison: 1 Rifampicin plus streptomycin (experimental) versus surgery alone (control), outcome: 1.1 Recurrence.

One prospective observational study, Kibadi 2010, examined rifampicin and streptomycin given for 12 weeks with surgery performed at week 4, in 92 participants with ulcerative lesions measuring more than 10 cm in diameter. The study showed a high healing rate at week 12 (85/92, 92.4%) and low recurrence rate at 2 years (2/92, 2.2%).

Five prospective observational studies examined treatment with rifampicin and streptomycin for eight weeks (Chauty 2007; Sarfo 2010; Agbenorku 2011; Adu 2013; Beissner 2015). In one study all participants received surgery either during or after treatment (in this study surgery included debridement and skin grafting,

not just excision) (Agbenorku 2011); in one study a select group received surgery after assessment at week 4 and week 8 (Chauty 2007); and in three studies a select group of participants received after eight weeks of treatment (Sarfo 2010; Adu 2013; Beissner 2015).

- Where surgery was given to a select group participants, surgery rate differed among studies: 5% in Sarfo 2010, 27% in Beissner 2015, 52% in Chauty 2007, and 52% in Adu 2013.
- Four studies reported healing rates for all participants, regardless of whether they received surgery or not: 84.5% in Beissner 2015, 96.3% in Agbenorku 2011, 99.3% in Sarfo 2010 and 100% in Chauty 2007.
- Four studies reported healing rates for participants who received combination therapy alone: 48% at week 8 in Adu 2013, 48% after week 8 in Chauty 2007, 69.8% after minimum of 6 months follow-up in Beissner 2015, and 95% at 12 months in Sarfo 2010.
- Follow-up showed recurrence was unusual: 0% in Sarfo 2010 and Beissner 2015, 0.5% in Agbenorku 2011, and 1.4% in Chauty 2007.

Rifampicin combined with clarithromycin

See Table 3.

Two prospective observational studies (51 participants) evaluated the use of rifampicin and clarithromycin. Both studies included surgery, either to all participants or a select group. All participants were healed at 12 months.

Chauty 2011 evaluated rifampicin and clarithromycin for eight weeks in 30 participants with lesions measuring less than 10 cm in diameter. They reported a high healing rate at 12 months with no recurrence at 18 months (30/30, 100%). Half of the participants (50%) healed without any form of surgery; 11 participants (37%) healed with limited surgery including curettage of the lesion or a minor excision; and 4 participants (13%) healed with extensive surgery including major excision followed by skin grafting.

O'Brien 2012 evaluated rifampicin and clarithromycin with surgery in 21 participants and reported a high healing rate (100%) and no recurrence at one year. Duration of the regimen was determined by the attending physician.

Rifampicin with streptomycin initially, changing to rifampicin with clarithromycin in consolidation phase

See Table 3.

One RCT and one prospective observational study examined healing rates starting with rifampicin and streptomycin, and then swapping to rifampicin and clarithromycin, with surgery as indicated. Both studies only included participants with small lesions, and more than 90% of participants healed without surgery.

One RCT, BURULICO Study 2010, evaluated a regimen of rifampicin plus streptomycin for 4 weeks followed by rifampicin plus clarithromycin for 4 weeks in 151 participants with lesions measuring less than 10 cm in diameter. They compared this to the standard treatment at the time of eight weeks of rifampicin and streptomycin. Both groups achieved high healing rates at 12 months without surgery (a small number in each group had skin grafting): new regimen 68/75 (91%), standard regimen 73/76 (96%). There was no significant difference in healing rate or recurrence between the two groups (RR 0.94, 95% CI 0.87 to 1.03; not estimable due to 0 cases in both groups; Figure 5, Analysis 2.1; Figure 6; Analysis 2.2) or in healing time.

Figure 5. Forest plot of comparison: 2 Rifampicin combined with clarithromycin versus rifampicin combined with streptomycin in the consolidation phase, outcome: 2.1 Cure.

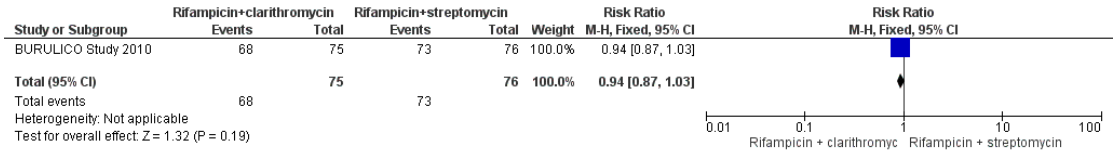
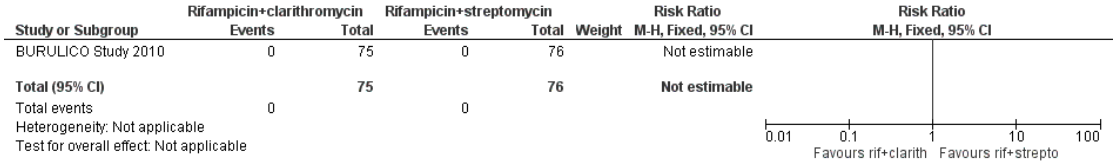


Figure 6. Forest plot of comparison: 2 Rifampicin plus clarithromycin (experimental) versus rifampicin plus streptomycin in the consolidation phase (control), outcome: 2.2 Recurrence at 12 months.



One prospective observational study, Phillips 2014a, evaluated a regimen of rifampicin plus streptomycin for 2 weeks followed by rifampicin plus clarithromycin for 6 weeks in 43 participants with lesions measuring less than 15 cm in diameter. Forty of 41 (98%) participants achieved healing by 52 weeks without surgery.

Novel combination therapy

See Table 4.

One RCT and two prospective observational studies investigated the efficacy of combinations of one to three drugs from the following: rifampicin, dapsone, ciprofloxacin, clarithromycin, moxifloxacin, ethambutol, amikacin, and azithromycin. High healing rates and low recurrence were achieved in the two prospective observational studies.

One RCT, Espey 2002, examined the efficacy of rifampicin and dapsone for 8 weeks against placebo in 30 participants with ulcerative lesions. No significant differences were observed for clinical improvement as judged by Buruli ulcer specialists using photographs (P = 0.51). A significant change in ulcer size after two months was observed (P = 0.02), however there was a significant difference in the initial ulcer size between the two groups.

Two prospective observational studies from the Australian group tested combinations of one to three oral antibiotics including rifampicin, ciprofloxacin, clarithromycin, moxifloxacin, ethambutol, amikacin, and azithromycin. Friedman 2016 evaluated participants who received the regimen with no surgery or with limited surgical debridement. Among the 160 participants in their registry, 28 participants (17.5%) who received extensive surgery were excluded, leaving 132 participants for their analysis. They reported that 131/132 (99%) participants healed at one year, among whom 101 (76.5%) participants healed with antibiotics alone. Median duration of antibiotic treatment was 56 days (interquartile range 24 to 96 days), and 22 participants (16.7%) needed fewer than 56 days to reach healing. O'Brien 2012 compared participants who were treated with antibiotics plus surgery to surgery alone. All 90/90 participants (100%) who underwent combined treatment with antibiotics plus surgery healed. Fourteen (30%) participants who received only surgery had recurrence. As the participants were retrieved from the same registry in these two studies, some participants may contribute data to more than one of the studies.

Adverse effects

Three RCTs evaluated adverse effects, of which two reported none (Espey 2002; Etuaful 2005). One RCT evaluated long-term adverse effects of streptomycin three to six years after treatment (BURULICO Study 2010). Among those that could be retrieved from the past BURULICO study (n = 127), ototoxicity was observed in 23% of adults in the 4-week streptomycin group and 40% of adults in the 8-week streptomycin group (total n = 41), and in 28% of children in the 4-week streptomycin group and 26% of children in the 8-week streptomycin group (total n = 86). Nephrotoxicity during treatment was observed in 9% of adults in the 4-week streptomycin group and 20% of adults in the 8-week streptomycin group, and in 5% of children in the 4-week streptomycin group and 20% of children in the 8-week streptomycin group. At long-term follow-up, one adult (2.4%) and two children (2.4%) were classified as having long-term nephrotoxicity, all from the 8-week streptomycin group.

Eight prospective observational studies evaluated adverse effects, of which two reported none (Chauty 2007 (rifampicin, streptomycin) and Agbenorku 2011 (rifampicin, streptomycin)). One study reported no discontinuation of antibiotics (rifampicin, clarithromycin) due to adverse effects (Chauty 2011). Lunn 1964 reported one participant with gastrointestinal intolerance from clofazimine. Sarfo 2010 reported one participant with dizziness and one with vomiting and dizziness from streptomycin, and one participant with rash probably from rifampicin. Phillips 2014a reported one participant with ototoxicity from streptomycin. O'Brien 2012 reported that of 90 participants who received antibiotic treatment, 28 (31%) developed adverse effects including gastrointestinal intolerance, hepatitis, rash, hypoglycaemia, joint or tendon effects, palpitations, and hallucinations. Friedman 2016 reported that 21 of the 132 participants (16%) developed adverse effects (unspecified) that required cessation of one or more antibiotics during treatment.

Paradoxical reactions

See Table 5.

Six prospective observational studies evaluated paradoxical reactions (Sarfo 2010; O'Brien 2012; O'Brien 2013b; Phillips 2014a;

Barogui 2016; Friedman 2016), of which two studies evaluated solely this outcome (O'Brien 2013b; Barogui 2016).

The incidence of paradoxical reactions ranged from 1.9% in Sarfo 2010 to 26% in Friedman 2016. Median onset time of paradoxical reactions ranged from 5.6 weeks (39 days) in O'Brien 2013b to 12 weeks in Phillips 2014a. As the participants were retrieved from the same registry in three studies (O'Brien 2012; O'Brien 2013b; Friedman 2016), some participants may contribute data to more than one of the studies.

ADDITIONAL SUMMARY OF FINDINGS *[Explanation]*

Rifampicin with clarithromycin compared with rifampicin with streptomycin in the consolidation phase for Buruli ulcer

Patient or population: people with Buruli ulcer, early lesions measuring less than 10 cm in diameter, aged 5 years or older
Settings: Ghana
Intervention: rifampicin with streptomycin, followed by rifampicin with clarithromycin after 4 weeks
Comparison: rifampicin with streptomycin continued

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	Number of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Streptomycin continued	Clarithromycin substitute				
Cure[a]	96 per 100	90 per 100 (84 to 99)	RR 0.94 (0.87 to 1.03)	151 (1 trial)	⊕⊕◯◯ LOW[b] due to imprecision	We do not know if the treatment is superior to the control.

*The basis for the **assumed risk** (for example, the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% CI) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
Abbreviations: CI: confidence interval; RR: risk ratio

GRADE Working Group grades of evidence
High certainty: further research is very unlikely to change our confidence in the estimate of effect.
Moderate certainty: further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low certainty: further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low certainty: we are very uncertain about the estimate.

[a]Cure is defined as "healing of skin lesions without recurrence at 12 months or longer." There were no recurrences in this study.
[b]Downgraded by 2 for imprecision: very few events and wide CIs.

DISCUSSION

Summary of main results

See Summary of findings for the main comparison and Summary of findings 2.

We included 18 studies, of which five were RCTs, in this review. Earlier studies conducted before 2000 that assessed monotherapy (clofazimine, sulfamethoxazole/trimethoprim) demonstrated no treatment effect. The remaining studies assessed combination therapy with or without surgery. The main regimens included rifampicin plus streptomycin, rifampicin plus clarithromycin, and rifampicin plus streptomycin switching to rifampicin plus clarithromycin during the consolidation phase.

It is evident that antimicrobials are important in treating Buruli ulcers; this was an already established fact, but also learned from this review. Different combinations of antibiotics are given for eight weeks to treat Buruli ulcer, irrespective of the stage. However, there were insufficient studies and data to be able to determine which regimen is the most effective. In 2004, the WHO first recommended a combination of rifampicin and streptomycin for eight weeks (WHO 2014). However, there is no evidence from RCTs to support this treatment. Five prospective observational studies tested this regimen, which reported healing rates from 84.5% to 100% with or without surgery. Four studies reported healing rates for participants who received combination therapy alone to be from 48% to 95%. The time points assessed in the studies varied, and therefore a comparison or calculation of a combined healing rate was not possible.

There has recently been movement from the current regimen, which requires injection, to an all-oral treatment, with the goal of reducing the burden of treatment for patients. Of the studies included in this review, BURULICO Study 2010 was the only RCT with adequate methods. This study tested rifampicin plus streptomycin for four weeks followed by rifampicin and clarithromycin for four weeks against rifampicin plus streptomycin for eight weeks, so that the patients will receive fewer injections of streptomycin. The study showed that there was no significant difference in healing rate and time between the two regimens. Other studies have investigated different combinations of oral drugs, with most regimens yielding high healing rates (Chauty 2011; O'Brien 2012; Friedman 2016). The study sample sizes were small, and their study design was weak to examine the effects of these regimens, however these studies show the potential of all-oral treatments. The WHO currently lists use of rifampicin (10 mg/kg once daily) with either streptomycin (15 mg/kg once daily) or clarithromycin (7.5 mg/kg twice daily) for eight weeks as the treatment choices for Buruli ulcer, depending on the patient (WHO 2012; WHO 2017).

When assessing the efficacy of treatments for Buruli ulcers, lesion size, lesion type, and whether surgery was applied or not are important factors to be considered. We attempted to perform a subanalysis, but this was not possible due to the heterogeneity of studies. It may also be important to consider the impact of the severity of lesions (WHO category) on treatment efficacy, however not all studies reported these data. It is important to note that some studies that reported high healing rates recruited only participants with small lesions, which may be important to consider when interpreting the results from these studies (Etuaful 2005; BURULICO Study 2010; Chauty 2011; Phillips 2014a).

Six prospective observational studies measured incidence of paradoxical reactions, which ranged from 1.9% to 26%. The pathogenesis of paradoxical reactions remains unclear, but recent studies report a possible association with antibiotic treatment and types of antibiotics used (O'Brien 2009; Nienhuis 2012; O'Brien 2013b).

Overall completeness and applicability of evidence

All studies included both males and females. With regards to age, participants from African countries were younger compared to those from Australia, which could have influenced the results. This is reflected by the different age distributions of the affected population between the two areas (Asiedu 1998; Wansbrough-Jones 2006; Boyd 2012). Comorbidities (including HIV) in participants were uncommon, or those with comorbidities were excluded from the study, with the exception of the Australia group studies. Rates of comorbidities in the two Australian studies (9.5% and 16.3%) could have affected their study results.

Five studies (26%) diagnosed Buruli ulcer based only on clinical presentation, otherwise all studies had laboratory confirmation of Buruli ulcer either by Ziehl-Neelsen test for AFB, polymerase chain reaction (PCR), or histopathology. All recent studies (after 2007) had laboratory confirmation of Buruli ulcer as part of their inclusion criteria.

Treatment was often given for eight weeks, which has been the WHO recommendation since 2004, and different durations were not tested. Dosages of the drugs were the same between studies: 10 mg/kg/day for rifampicin, 15 mg/kg/day for streptomycin, and 7.5 mg/kg/day for clarithromycin. Intervention with surgery made it a challenge to compare the outcomes between studies. However, it is an important adjunctive intervention to drugs for treating Buruli ulcer, and participants who received surgery were included in the study results. The extent/definition of surgical intervention differed between studies: for example, skin grafting was not considered to be surgery in BURULICO Study 2010 and Friedman 2016. Furthermore, the decision of when to intervene with surgery differs among surgeons/clinicians, and this may have affected the results. It is also important to note that earlier studies tended to perform surgery more often than current studies, as it used to be the standard treatment.

Healing as defined by complete epithelialization was the primary outcome in most studies, but not in the earlier studies, where it was change in ulcer size (Fehr 1994; Espey 2002; Etuaful 2005). The only other outcome that was comparable between studies

was recurrence. Assessment time points differed between studies, which made it impossible to compare or synthesize the results from different studies.

Certainty of the evidence

The certainty of the evidence was very low.

Potential biases in the review process

We attempted to limit bias in the review process. Vittoria Lutje, the Cochrane Infectious Diseases Group Information Specialist, conducted the literature searches, and it is unlikely that these searches missed any major studies; however, we cannot rule out the possibility that we missed some small unpublished studies.
We included prospective observational studies in this review as there was a very limited number of RCTs investigating this topic. This decision was made after a number of discussions between the authors, the Cochrane Infectious Diseases Group, and their reviewers. While this may have created some bias in this review, we have tried to minimize bias by reporting the results of prospective observational studies separately from RCTs.
To limit bias in the study selection process and data extraction, we independently examined the search results, selected studies, and extracted data.

Agreements and disagreements with other studies or reviews

There were no other studies or reviews with which to compare this review.

AUTHORS' CONCLUSIONS

Implications for practice

People with Buruli ulcers should receive drug therapy. This is obvious and not the subject of this Cochrane Review.

Regarding choice of drug and duration of treatment, the studies included in this review did not provide substantive evidence to guide recommendations. The differences in efficacy between drugs is still uncertain, although the included studies at low risk of bias did demonstrate a high healing rate of Buruli ulcer lesion(s) with the use of combined drug therapy, with or without surgery. How different sizes/lesions/stages of the disease may contribute to healing and which kind of lesions are in need of surgery were unclear from the included studies. These factors need to be considered, as does the practicality of the treatment in resource-limited settings where most people with Buruli ulcer reside, when guiding recommendations for the treatment of Buruli ulcer.

The current available evidence does not support the use of rifampicin and streptomycin for an eight-week duration, which has been the standard regimen used in endemic African countries.

Implications for research

There were two ongoing trials at the time of publication of this Cochrane Review. One trial is a multicentre study between Ghana and Benin testing rifampicin and clarithromycin for eight weeks (NCT01659437). The other trial is testing the timing of surgery with rifampicin and streptomycin for eight weeks (NCT01432925). The preliminary results of these studies were reported at the World Health Organization Buruli Ulcer Meeting in March 2017 and will be included in our updated review.

Conducting field trials to test treatment effect of Buruli ulcer is complex and challenging, as:

- there are a limited number of patients (2000 to 5000 annual cases globally); and
- there are multiple treatments that contribute to healing including surgery and wound care and not just drugs.

Although we assessed the certainty of the evidence of the studies included in this review as low, researchers have worked hard to generate this body of evidence under these circumstances.

Further research will be useful testing different regimens, including the possibility of new drugs/combinations; different durations of treatment depending on the lesion stage; and timing of surgical interventions. Antituberculosis drugs that arrived recently on the market could also be useful in the treatment of Buruli ulcer and need to be tested, yet the high cost of these drugs is a concern for use in resource-limited settings, where most patients reside. Cost analysis of treatment - which is often neglected and needs more attention - is therefore also an important area for investigation. As Buruli ulcer is a toxic disease while it is an infection, antitoxins or other systemic drugs may bring about a breakthrough in the treatment of the disease and are interesting areas for exploration. The development of reliable and low-cost point-of-care diagnostic tools are needed to promote a better body of evidence for Buruli ulcer treatment. The primary diagnostics to confirm the disease is currently polymerase chain reaction (PCR), which is not readily available in many endemic areas. Assessment time points of healing and recurrence should be made uniform so as to allow comparison between studies, and such action should be initiated. This can be facilitated by the development of tools to quantify healing. For example, level of mycolactone in lesions, blood, or urine could be a candidate for this purpose in the future when quantitative test of mycolactone will be made easier to use. Wound care is another essential focus for research in Buruli ulcer, which may also benefit

other diseases with ulcers. Operational research in order to detect, diagnose, and treat patients early also needs to be promoted.

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* *Indicates the major publication for the study*

CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Adu 2013

Methods	Prospective observational study
Participants	Inclusion criteria: clinically + laboratory-confirmed BU (exclusion: none stated) Laboratory confirmation: either by ZN test for acid-fast bacilli (AFB), PCR, or histopathology Enrolled: 126 participants Participant characteristics: 64 males, 50.8%; mean age 29.8 (range, 1 year 3 months to 98 years) Lesion types: ulcer 116 (92%), papule 1 (0.5%), nodule 2 (1.5%), oedema 4 (3%), chronic osteomyelitis 2 (1.5%), contractures 2 (1.5%) (1 participant with both ulcer and contracture) WHO category I: 12 (10%), category II: 43 (34%), category III: 71 (56%)
Interventions	Rifampicin (10 mg/kg/day) + streptomycin (15 mg/kg/day), 8 weeks, with surgery Surgery: when indicated after antibiotic treatment Follow-up: N/A
Outcomes	"Healed without surgery", assessed at 8 weeks **Standardized outcome: possible cure**
Notes	Trial location: Ghana Enrolment dates: January 2010 to December 2012 The primary objective of the study was to document the complications of BU and the reconstructive surgery performed in patients whose lesions were not completely healed after 8 weeks of antibiotic treatment

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (Trials)	High risk	-
Allocation concealment (Trials)	High risk	-
Blinding of participants and personnel (Trials)	High risk	-
Blinding of outcome assessment (Trials)	High risk	-
Selection of participants into the study (Prospective observational studies)	Low risk	Selection not related to intervention or outcome.

Adu 2013 (*Continued*)

Measurement of outcomes (Prospective observational studies)	Low risk	Objective outcome measure (healing)
Incomplete outcome data / missing data (All studies)	Low risk	No missing data
Selective reporting (All studies)	Low risk	Reported all expected outcomes
Other bias	Low risk	No other bias identified.

Agbenorku 2011

Methods	Prospective observational study
Participants	Inclusion criteria: clinically + laboratory-confirmed BU (exclusion: none stated) Laboratory confirmation: any 2 positives of ZN test for AFB, PCR, and histopathology Enrolled: 189 participants Participant characteristics: 113 males, 60%; age N/A Lesion types: ulcer 145 (76.7%), nodule 38 (20.1%), plaque 6 (3.2%) WHO category I: 44 (22.3%), categories II + III: 145 (76.7%)
Interventions	Rifampicin (10 mg/kg/d) + streptomycin (15 mg/kg/d), 8 weeks, with surgery Surgery: all cases Follow-up: 2 years after discharge from hospital
Outcomes	1. Healing rate 2. Recurrence 3. Adverse effects 4. Mean hospital stay days 5. Number of new BU cases and their disease stage at the study site after counselling and health education activities **Standardized outcome: cure**
Notes	Trial location: Ghana Enrolment dates: January 2005 to December 2005

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (Trials)	High risk	-
Allocation concealment (Trials)	High risk	-
Blinding of participants and personnel (Trials)	High risk	-

Agbenorku 2011 (*Continued*)

Blinding of outcome assessment (Trials)	High risk	-
Selection of participants into the study (Prospective observational studies)	Low risk	Selection not related to intervention or outcome.
Measurement of outcomes (Prospective observational studies)	Low risk	Objective outcome measure (healing)
Incomplete outcome data / missing data (All studies)	Low risk	3 participants (3.2%) were lost to follow-up but for different outcomes
Selective reporting (All studies)	Low risk	Reported all expected outcomes
Other bias	Low risk	No other bias identified.

Barogui 2016

Methods	Prospective observational study
Participants	Inclusion and exclusion criteria: refer to BURULICO Study 2010 and NCT01432925 Enrolled: 241 participants; 150 from BURULICO Study 2010 and 91 from NCT01432925 Participant characteristics: 88 (37%) males, mean (SD) 16.2 (13.2) years Lesion types: ulcer 108 (45%), nodule 32 (13%), plaque 56 (23%), oedema 11 (5%), mixed 34 (14%) WHO category I: 69 (29%), category II: 133 (55%), category III: 39 (16%)
Interventions	Refer to BURULICO Study 2010 and NCT01432925
Outcomes	Paradoxical reaction defined by an initial decrease of the lesion size followed by 2 consecutive increases
Notes	Trial location: Ghana and Benin Enrolment dates: BURULICO Study 2010, 2006 to 2008; NCT01432925, 2011 to 2015

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (Trials)	High risk	-
Allocation concealment (Trials)	High risk	-
Blinding of participants and personnel (Trials)	High risk	-

Barogui 2016 (*Continued*)

Blinding of outcome assessment (Trials)	High risk	-
Selection of participants into the study (Prospective observational studies)	Low risk	Selection not related to intervention or outcome.
Measurement of outcomes (Prospective observational studies)	Low risk	Objective outcomes clearly defined.
Incomplete outcome data / missing data (All studies)	Low risk	No loss to follow-up
Selective reporting (All studies)	Low risk	Reported all expected outcomes
Other bias	High risk	Paradoxical reaction was only defined clinically by lesion size; no exams to support diagnosis

Beissner 2015

Methods	Prospective observational study
Participants	Inclusion criteria: clinically + laboratory-confirmed BU; any age (exclusion: laboratory-unconfirmed cases) Laboratory confirmation: IS2404 dry-reagent-based PCR Enrolled: 199 eligible participants; 70 dropouts (35.2%); 129 participants analysed Participant characteristics: 60 males, 46.5%; median 10 years, range 2 to 68 years Lesion types: ulcer 73 (76.7%), nodule 19 (14.7%), plaque 26 (20.2%), oedema 11 (8.5%) WHO category I: 59 (45.7%), category II: 44 (34.1%), category III: 26 (20.2%)
Interventions	Rifampicin (10 mg/kg/d) + streptomycin (15 mg/kg/d), 8 weeks Surgery: when indicated Follow-up: follow-up visits were conducted January to April 2013 (110 participants) and May to June 2014 (19 participants) (> 6 months elapsed since the end of antimicrobial treatment)
Outcomes	1. Healed with or without surgery 2. Healing time 3. Secondary lesions 4. Functional limitations 5. Recurrence until the follow-up visit **Standardized outcome: possible cure**
Notes	Trial location: Togo Enrolment dates: September 2007 to November 2013
Risk of bias	

Beissner 2015 (*Continued*)

Bias	Authors' judgement	Support for judgement
Random sequence generation (Trials)	High risk	-
Allocation concealment (Trials)	High risk	-
Blinding of participants and personnel (Trials)	High risk	-
Blinding of outcome assessment (Trials)	High risk	-
Selection of participants into the study (Prospective observational studies)	High risk	Only 129 out of 199 eligible patients could be retrieved and enrolled
Measurement of outcomes (Prospective observational studies)	Low risk	Objective outcome measure (healing)
Incomplete outcome data / missing data (All studies)	Low risk	No missing data
Selective reporting (All studies)	Low risk	Reported all expected outcomes
Other bias	Low risk	No other bias identified.

BURULICO Study 2010

Methods	Randomized controlled trial
Participants	Inclusion criteria: clinically + laboratory-confirmed BU; aged 5 years or older, had a reported disease duration of less than 6 months, and had lesions with a cross-sectional diameter (indurated area) of 10 cm or less (exclusions: pregnancy, drug intolerance, and renal, hepatic, and acoustic impairment) Laboratory confirmation: IS2404 dry-reagent-based PCR Enrolled: 151 participants; 143 with infection confirmed by PCR, 5 with infection confirmed by other methods, 3 cases were clinical diagnosis Participant characteristics: intervention group 19 (25%) males, median 12 years (IQR 9 to 22); control group 27 (36%) males, median 12 years (IQR 8 to 18) Lesion types: ulcer 59 (39.1%), non-ulcer 92 (60.9%) WHO category I: 58 (38.4%), category II + III: 93 (61.16%)
Interventions	1. Rifampicin (10 mg/kg/day) + streptomycin (15 mg/kg/day), 4 weeks followed by rifampicin (10 mg/kg/day) + clarithromycin (7.5 mg/kg/day), 4 weeks 2. Rifampicin (10 mg/kg/day) + streptomycin (15 mg/kg/day), 8 weeks Surgery: when indicated Follow-up: once a week participants were given study drugs to take to the nearest health facility to receive directly observed treatment for the subsequent days, with daily wound care. Participants with complicated lesions were hospitalized

	Participants were followed up at weekly intervals during the first 8 weeks; at week 10, week 12, and then monthly to week 36, and bimonthly to week 52. Study visits included clinical assessment with reporting of adverse effects, measurement of lesion size (if not healed) by tracing onto an acetate sheet, and photography of the lesion
Outcomes	1. Healed without surgery or recurrence (cure) 2. Cumulative proportion of healing 3. Difference in healing time between the 2 groups 4. Skin grafts 5. Recurrence 6. Functional impairment 7. Adverse effects (ototoxicity, nephrotoxicity, abdominal discomfort) during treatment 8. Long-term adverse effects (ototoxicity, nephrotoxicity) (Klis 2014)
Notes	Trial location: Ghana Enrolment dates: April 2006 to January 2008 HIV antibody testing was done with cold-stored sera after completion of the study, in which 3 (2%) participants were found positive

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (Trials)	Low risk	Computer-generated minimization
Allocation concealment (Trials)	Low risk	Assigned allocation was sent from a central site by text message to study co-ordinator
Blinding of participants and personnel (Trials)	Low risk	Open-label, but outcome unlikely to be affected by participant knowledge of treatment group
Blinding of outcome assessment (Trials)	Low risk	Open-label, but primary endpoint also assessed by blinded wound experts, and the results concurred with those from the primary analysis
Selection of participants into the study (Prospective observational studies)	Unclear risk	-
Measurement of outcomes (Prospective observational studies)	Unclear risk	-
Incomplete outcome data / missing data (All studies)	Low risk	4 participants withdrew/died/were lost to follow-up but were still included in analysis for primary endpoint as the lesion had healed at the last assessment
Selective reporting (All studies)	Low risk	Reported all expected outcomes

Other bias	Low risk	3 cases not laboratory-confirmed, but only a small number.

Chauty 2007

Methods	Prospective observational study
Participants	Inclusion criteria: clinical diagnosis of BU; residing in an endemic area (exclusion criteria: pregnancy; receiving traditional treatment at the time of diagnosis; history of leprosy, TB; liver, kidney, or hearing problems) Enrolled: 310 participants; 36 immediate surgical excision at another centre, 3 pregnancy, 47 refusals; 224 analysed Participant characteristics: among the 310 eligible participants, 145 males, 47%; < 15 years, 179 participants (58%), 15 to 49 years, 90 participants (29%), ≥ 50 years, 41 participants (13%) Lesion types: ulcer 168 (73.8%), of which 29 (18.4%) were < 5 cm, 76 (48.1%) were 4 to 14 cm, and 63 (39.9%) were ≥ 10 cm; non-ulcer 56 (26.2%) WHO category: N/A
Interventions	8 weeks of rifampicin (10 mg/kg/day) + streptomycin (15 mg/kg/day) was administered to all participants. During the 4-week assessment, participants who were considered unlikely to be cured by antibiotics alone (opinion of the treating physician) underwent surgery Local dressings were provided for participants with ulcerative lesions. Participants were treated daily under the direct observation of the clinic nurse either as an outpatient or inpatient. The study physician evaluated participants every 2 weeks during treatment Surgery: when indicated Follow-up period: 1 year after treatment completion
Outcomes	1. Successful treatment: completely healed 2. Recurrence: reactivation of the disease within 1 year after apparent success upon treatment completion **Standardized outcome: probable cure**
Notes	Trial location: Benin Enrolment dates: January 2003 to December 2004

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (Trials)	High risk	-
Allocation concealment (Trials)	High risk	-
Blinding of participants and personnel (Trials)	High risk	-

Chauty 2007 (*Continued*)

Blinding of outcome assessment (Trials)	High risk	-
Selection of participants into the study (Prospective observational studies)	High risk	86/310 patients (28%) who were eligible for the study were not included; 3 pregnancies, 36 immediate surgical excisions at another centre, 6 participants' decision to receive traditional treatment, 41 refusals of antibiotic and/or surgical treatment
Measurement of outcomes (Prospective observational studies)	Low risk	Objective outcome measure (healing)
Incomplete outcome data / missing data (All studies)	High risk	We do not have data for 17 participants who were lost to follow-up at week 8
Selective reporting (All studies)	Low risk	Reported all expected outcomes
Other bias	High risk	Laboratory exam was attempted in 145 of the 168 participants. 40/145 (28%) were negative for both PCR and smear, indicating that some cases may not have been BU

Chauty 2011

Methods	Prospective observational study
Participants	Inclusion criteria: clinically + laboratory-confirmed BU; at least 5 years of age, presented with lesions $\leq$ 10 cm in diameter that had appeared within the past 6 months, agreed to be hospitalized during treatment, and were likely to be followed up for 18 months (exclusion criteria: multiple lesions, lesions located over a joint, history of treatment with antimycobacterial drugs, receipt of macrolide or quinolone antibiotics during the previous month, allergy to rifampicin or clarithromycin, pregnancy, or HIV infection) Laboratory confirmation: IS2404 dry-reagent-based PCR Enrolled: 30 participants for analysis Participant characteristics: 12 males, 40%; 11 were > 15 years of age Lesion types: ulcer 21 (47%), non-ulcer 9 (30%) WHO category I: 13 (43%), category II: 17 (57%)
Interventions	Rifampicin (10 mg/kg/d) + clarithromycin (12 mg/kg/d), 8 weeks Surgery: when indicated Follow-up: participants were hospitalized during treatment for daily direct observation by nursing staff and were examined by a doctor every week. Swabs or aspiration samples were collected at week 4, 6, 8 if the lesion had not healed for culture and PCR. Participants were discharged from hospital when healed and were followed up every 3 months up to 18 months after start of treatment
Outcomes	1. Wound healing at 12 months, without recurrence 18 months after initiation of antibiotics 2. Need of additional care to antibiotics (limited surgery, excision and skin grafting)

Chauty 2011 (*Continued*)

	Standarized outcome: cure	
Notes	Trial location: Benin Enrolment dates: December 2007 to February 2009	
Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (Trials)	High risk	-
Allocation concealment (Trials)	High risk	-
Blinding of participants and personnel (Trials)	High risk	-
Blinding of outcome assessment (Trials)	High risk	-
Selection of participants into the study (Prospective observational studies)	High risk	2/3 of eligible population refused participation due to refusal of hospitalization
Measurement of outcomes (Prospective observational studies)	Low risk	Objective outcome measure (healing)
Incomplete outcome data / missing data (All studies)	Low risk	No missing data
Selective reporting (All studies)	Low risk	Reported all expected outcomes
Other bias	Low risk	No other bias identified.

Espey 2002

Methods	Randomized controlled trial
Participants	Inclusion criteria: clinical diagnosis of BU*; aged 4 years or older (excluded: history of TB, leprosy, HIV, liver disease, pregnancy, breastfeeding) Case definition: the presence of a painless or minimally painful cutaneous ulcer with undermined margins Enrolled: 41 participants; 10 participants were lost to follow-up, 1 pregnancy: 30 participants for analysis Participant characteristics: intervention group 11 (73%) males, median 13 years old (range, 5 to 60); control group 7 (47%) males, median 10 years old (range, 5 to 60) Lesion types: ulcer 30 (100%) WHO category: N/A

Espey 2002 (*Continued*)

Interventions	1. Dapsone (1.0 to 1.5 mg/kg/day) + rifampicin (10 to 20 mg/kg/day), 2 months 2. Placebo identical in appearance to dapsone, 2 months Both groups received supportive local ulcer care consisting of cleansing and non-surgical debridement Surgery: none Follow-up: 2 months; photographs were taken at enrolment and at 2 months for 28 participants
Outcomes	1. Clinical change, judged by photographs as "worse", "unchanged", or "improved", by 2 specialists blinded to group 2. Change in ulcer size 3. Adverse effects
Notes	Trial location: Côte d'Ivoire Enrolment dates: 3 March to 4 April 1994 *Diagnostic tests: not all cases were confirmed as BU: 6/41 (14.6%) skin biopsies were diagnostic of BU (containing AFB or active necrosis of adipose tissue); 29 (70.7%) were indicative of BU (granulomatous changes and necrosis, without AFB); the remaining 3 revealed non-specific inflammation; 3/41 (7%) yielded positive cultures

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (Trials)	Low risk	Quote: "Participants were randomized by lot to one of two groups."
Allocation concealment (Trials)	Unclear risk	No information on allocation concealment
Blinding of participants and personnel (Trials)	Low risk	It does not seem that participants were blinded fully, as the placebo participants received only 1 pill, whereas the treatment group participants received 2 pills. Investigators were also not blinded. However, outcome is probably unlikely to be influenced by lack of blinding
Blinding of outcome assessment (Trials)	High risk	Investigators not blinded to treatment status, and outcome is likely to have been affected by lack of blinding
Selection of participants into the study (Prospective observational studies)	Unclear risk	-
Measurement of outcomes (Prospective observational studies)	Unclear risk	-
Incomplete outcome data / missing data (All studies)	High risk	10 participants were lost to follow-up with no reasons provided. Given this study's sample size, this is a relatively large amount of missing data. The numbers in each group were initially balanced across treatment and

Espey 2002 (*Continued*)

		placebo groups
Selective reporting (All studies)	Low risk	Reported all expected outcomes
Other bias	High risk	There is a big difference in initial median ulcer size between treatment and placebo groups Photography outcome is prone to subjectivity. A total of 41 skin biopsies from 30 enrolled participants were taken. 3/30 (10%) yielded no specific change compatible to BU, indicating these cases may not be BU

Etuaful 2005

Methods	Randomized controlled trial
Participants	Inclusion criteria: clinically + laboratory-confirmed BU; had a single nodule or plaque ≤ 10 cm in maximum diameter; aged 15 years or older (exclusion criteria: pregnancy, treatment with antibiotics, history of leprosy, TB, liver, kidney, or hearing problems) Laboratory confirmation: either 1 or 2 1. Culture (+) or definite histopathology (the presence of Buruli-type coagulative necrosis of the dermis or subcuticular issue, with or without granulomas, and with or without AFB) 2. PCR (+) plus possible histopathology (the presence of panniculitis, with or without granulomas but without Buruli-type coagulative necrosis) or visible AFB Enrolled: 33 participants; 1 participant withdrew, 1 excluded, 1 resolved completely during treatment, 9 participants did not meet final laboratory diagnosis; 21 participants for analysis Participant characteristics: 7 male, 33%; mean age N/A Lesion types: 14 nodules, 7 plaques WHO category: N/A
Interventions	5-arm study comparing immediate excision of the lesion and closure of the wound (no antibiotic), rifampicin (10 mg/kg/d) + streptomycin (15 mg/kg/d) given for 4 weeks, 8 weeks, 12 weeks before excision of the lesion Additional arm added during the study of rifampicin (10 mg/kg/d) + streptomycin (15 mg/kg/d), 2 weeks before excision of the lesion All participants were admitted to hospital for directly observed therapy Surgery: all cases Follow-up period: 12 months after surgery
Outcomes	1. Change in mean surface areas of lesions before and after treatment with antibiotics for 2, 4, 8, or 12 weeks 2. Recurrence 3. Adverse effects
Notes	Trial location: Ghana Enrolment dates: September 2001 to December 2002

Etuaful 2005 (*Continued*)

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (Trials)	High risk	Participants in 4 groups (4 week, 8 week, 12 week, and surgery only) were randomized using computer-generated numbers. Participants in 2 week treatment group were recruited sequentially and were not randomized
Allocation concealment (Trials)	Unclear risk	No information on allocation concealment
Blinding of participants and personnel (Trials)	Low risk	Blinding was not possible given different lengths of treatment and surgical intervention, however outcome is unlikely to be influenced by lack of blinding
Blinding of outcome assessment (Trials)	Unclear risk	No information on blinding of assessors, and outcome may be affected by lack of blinding
Selection of participants into the study (Prospective observational studies)	Unclear risk	-
Measurement of outcomes (Prospective observational studies)	Unclear risk	-
Incomplete outcome data / missing data (All studies)	Low risk	All 21 participants eligible for analysis in the study were analysed
Selective reporting (All studies)	Low risk	Reported all expected outcomes
Other bias	Low risk	No other bias identified.

Fehr 1994

Methods	Randomized controlled trial
Participants	Inclusion criteria: clinically + laboratory-confirmed BU (exclusion criteria: none stated) Laboratory confirmation: diagnosis of BU was confirmed by ZN staining in 6 participants, culture in 4 participants, and histopathology in the remaining participants Enrolled: 18 participants; 6 excluded from analysis due to lost to follow-up (4) and death (2); 12 participants for analysis Participant characteristics: 5 male, 42%; sulfamethoxazole/trimethoprim group: 18.3 years (5 to 32), placebo: 20.8 years (8 to 45) Lesion types: ulcer 12/12 (100%) 1. 4/6 (66%) in the sulfamethoxazole/trimethoprim and 2/6 (33%) in the placebo group received surgery prior to intervention. 2. Initial ulcer size was 73.8 (9 to 247) cm^2 for the sulfamethoxazole/trimethoprim and 38.7 (15 to 80) cm^2 for the placebo group.

Fehr 1994 (*Continued*)

Interventions	1. Sulfamethoxazole/trimethoprim (800 mg sulfamethoxazole/160 mg trimethoprim) 1 tablet twice daily for participants above 12 years; 0.5 tablet twice daily for younger participants 2. Identical placebo Surgery: when indicated Follow-up: follow-up was done by standardized examination including photographic documentation of ulcer size by the same observer up to 5 times in approximately 2-weekly intervals
Outcomes	1. Percentage change of ulcer size* at study end 2. Percentage covered by granulation tissue at study end 3. Excision during follow-up *Ulcer size: calculated by multiplying the greatest width by the greatest depth in centimetres The study was concluded whenever further excision became necessary during follow-up
Notes	Trial location: Ghana Enrolment dates: February to June 1988

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (Trials)	High risk	Used alternate allocation method
Allocation concealment (Trials)	Unclear risk	Unclear statement: "Patients were, in double-blinded fashion, alternately allocated to Batrium forte or identical placebo."
Blinding of participants and personnel (Trials)	Low risk	Identical placebo was used.
Blinding of outcome assessment (Trials)	Unclear risk	Not explicitly stated that the outcome assessor was blinded, though outcome was assessed by 1 individual
Selection of participants into the study (Prospective observational studies)	Unclear risk	-
Measurement of outcomes (Prospective observational studies)	Unclear risk	-
Incomplete outcome data / missing data (All studies)	Low risk	Proportion of missing data is relatively large considering the sample size. However, reasons for exclusions/missing data are relatively well balanced or unlikely to be related to true outcome
Selective reporting (All studies)	Low risk	Reported all expected outcomes

Fehr 1994 (*Continued*)

Other bias	High risk	Baseline characteristics are not well balanced. No significant differences, but the groups are so small the P values would not detect significant differences

Friedman 2016

Methods	Prospective observational study
Participants	Inclusion criteria: clinically + laboratory confirmed; treated with antimicrobials alone or with limited surgical debridement* (excluded: those who underwent extensive surgery**) *Curettage of the lesion or a minor excision to remove excess granulation tissue and to debride ulcer margins, with or without use of a split skin graft **Complete excision of the entire lesion including margins of non-necrotic tissue, with either direct closure or the use of a split skin graft or a vascularized skin and tissue flap for reconstruction or to cover the defect Laboratory confirmation: any of (1) a culture of *Mycobacterium ulcerans* from the lesion, (2) PCR(+), or (3) histopathology showing a necrotic granulomatous ulcer with the presence of AFB Enrolled: 160 participants; 28 underwent extensive surgery and were excluded; 132 participants for analysis Participant characteristics: 75 males, 56.8%; median age 49 years (range, 1 to 95) Lesion types: ulcer 110 (83.3%), nodule 9 (6.8%), oedema 10 (7.6%), plaque 3 (2.3%) WHO category I: 104 (78.8%), category II: 19 (14.4%), category III: 9 (6.8%)
Interventions	Antibiotics alone or antibiotics with limited surgical debridement Included regimens: Rifampicin (10 mg/kg/d) plus • Ciprofloxacin (500 mg twice daily) • Clarithromycin (500 mg twice daily; 7.5 to 15 mg/kg/daily in divided doses in children) • Moxifloxacin (400 mg daily) Surgery: when indicated Follow-up: 12 months
Outcomes	1. Treatment success defined as complete healing of the *M ulcerans* lesion without recurrence within 12 months of treatment commencement **(cure)** 2. Recurrence 3. Treatment failure 4. Adverse effects 5. Paradoxical reactions 6. Duration of antibiotic administration
Notes	Trial location: Australia Enrolment dates: 1 October 2010 to 31 December 2014 13/132 participants (9.5%) had diabetes mellitus.
Risk of bias	

Friedman 2016 (*Continued*)

Bias	Authors' judgement	Support for judgement
Random sequence generation (Trials)	High risk	-
Allocation concealment (Trials)	High risk	-
Blinding of participants and personnel (Trials)	High risk	-
Blinding of outcome assessment (Trials)	High risk	-
Selection of participants into the study (Prospective observational studies)	Low risk	All patients who met the study inclusion criteria were included
Measurement of outcomes (Prospective observational studies)	Low risk	Objective outcome measure (healing)
Incomplete outcome data / missing data (All studies)	Low risk	No missing data
Selective reporting (All studies)	Low risk	Reported all expected outcomes
Other bias	Unclear risk	9.5% of participants had comorbidities that may have affected healing rate and time

Kibadi 2010

Methods	Prospective observational study
Participants	Inclusion criteria: clinically diagnosed BU; ulcerative lesions with maximum diameter ≥ 10 cm; 3 to 75 years old; residence in an endemic area (exclusion criteria: previous treatment by rifampicin or streptomycin; previous diagnosis of leprosy or TB; pregnancy; presence of cardiovascular, hepatic, or renal disease) Enrolled: 94 participants; 1 refusal, 1 lost to follow-up; 92 participants for analysis Participant characteristics: 43 males, 47%; 38 participants ≤ 15 years, 43 participants 15 to 49 years, 11 participants ≥ 50 years Lesion types: ulcer 92 participants (100%) WHO category II: 90 participants (97.8%), category III: 2 participants (2.2%)
Interventions	Rifampicin (10 mg/kg/d) + streptomycin (15 mg/kg/d) for 12 weeks, with surgery after the first 4 weeks Local treatment was applied daily with an aqueous solution of chloramine-metronidazole-nitrofurandoine Surgery: all cases Follow-up period: follow-up evaluation was carried out at the end of the 4th and 12th week of treatment. Recurrence was followed up for at least 2 years after treatment completion

Outcomes	1. Clinical outcome: "success" (4th week: 10% to 30% reduction in ulcer size and/or absence of new necrotic tissue, 12th week: healed), "clinical status quo" (no change in the size or presence of necrotic tissue), or "failure" (increase in the size and presence of new necrotic tissue) 2. Recurrence: reappearance of an ulcer or another form of the disease (nodule, papule, plaque, oedema, or bone involvement) at the original site of the lesion or elsewhere **Standardized outcome: cure**
Notes	Trial location: Democratic Republic of Congo Enrolment dates: October 2006 to September 2007 Clinical outcome was compared between PCR(+) and PCR(-) participants PCR(+): 61 participants PCR(-): 31 participants

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (Trials)	High risk	-
Allocation concealment (Trials)	High risk	-
Blinding of participants and personnel (Trials)	High risk	-
Blinding of outcome assessment (Trials)	High risk	-
Selection of participants into the study (Prospective observational studies)	Low risk	Selection not related to intervention or outcome.
Measurement of outcomes (Prospective observational studies)	Low risk	Objective outcome measure (healing)
Incomplete outcome data / missing data (All studies)	Low risk	1 lost to follow-up, 1 death
Selective reporting (All studies)	Low risk	Reported all expected outcomes
Other bias	High risk	PCR-negative cases (30 participants, 33%) may not be BU.

Lunn 1964

Methods	Prospective observational study
Participants	Inclusion criteria: individuals with proved mycobacterial ulceration who presented themselves for treatment (exclusion criteria: none stated) Enrolled: 10 participants Participant characteristics: 6 males, 60%; median age 12.7 years (range, 6 to 20) Lesion type: ulcers 10/10 (100%) WHO category: N/A
Interventions	B.663 (riminophenazine derivative; currently, clofazimine) was given as part of preparation for operation for 1 to 4 weeks and continued after operation until healing Adults > 50 kg: 300 mg/day Adults 25 to 50 kg, children: 200 mg/day Surgery: all cases Follow-up: not specified
Outcomes	Healing **Standarized outcome: possible cure**
Notes	Trial location: Uganda Enrolment dates: none stated.

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (Trials)	High risk	-
Allocation concealment (Trials)	High risk	-
Blinding of participants and personnel (Trials)	High risk	-
Blinding of outcome assessment (Trials)	High risk	-
Selection of participants into the study (Prospective observational studies)	Low risk	Selection not related to intervention or outcome.
Measurement of outcomes (Prospective observational studies)	Low risk	Objective outcome measure (healing)
Incomplete outcome data / missing data (All studies)	Unclear risk	Not clear at what time point participants were assessed and whether they had data for all 10 participants at a given time point
Selective reporting (All studies)	Unclear risk	No predefined outcomes
Other bias	Unclear risk	No laboratory confirmation

O'Brien 2012

Methods	Prospective observational study
Participants	Inclusion criteria: clinically + laboratory-confirmed BU; managed with surgery (exclusion criteria: none stated) Laboratory confirmation: any of (1) a culture of *Mycobacterium ulcerans* from the lesion, (2) PCR(+), or (3) histopathology showing a necrotic granulomatous ulcer with the presence of AFB Enrolled: 147 participants; 1 without surgery, 2 deaths, 1 lost to follow-up, 10 ongoing treatment were excluded; 137 lesions of 133 participants analysed Participant characteristics: 67 males, 50.4%; median age 62 years (range, 3 to 94) Lesion types: clinical type of lesion was recorded in 122/133 participants (92%); ulcer 106 (87%), nodules 9 (7%), oedematous lesion 7 (6%) WHO classification: N/A
Interventions	Surgery with or without different oral antibiotic treatments 90 participants received antibiotics as follows. • Rifampicin + ciprofloxacin (55 participants, 61%) • Rifampicin + clarithromycin (21 participants, 23%) • Rifampicin + clarithromycin, and ethambutol (5 participants, 4%) • Ciprofloxacin + clarithromycin (4 participants, 4%) • Rifampicin + moxifloxacin (2 participants, 2%) • Clarithromycin + ethambutol (1 participant, 1%) • Rifampicin + ethambutol, and amikacin (1 participant, 1%) • Clarithromycin only (1 participant, 1%) Drug dosages: • Rifamipicin 10 mg/kg/day (up to a maximum of 600 mg/day) • Ciprofloxacin 500 mg twice daily • Clarithromycin 500 mg twice daily • Moxifloxacin 400 mg daily • Amikacin 15 mg/kg/day • Ethambutol not given Surgery: all cases Follow-up: 12 months
Outcomes	1. Treatment success, defined as complete healing of the *M ulcerans* lesion without recurrence within 12 months of treatment commencement **(cure)** 2. Recurrence 3. Antibiotic duration prior to surgery 4. Adverse effects 5. Paradoxical reactions
Notes	Trial location: Australia Enrolment dates: March 1998 to May 2010 11 participants were complicated with diabetes mellitus, 5 with malignancy, 4 with connective tissue disease, and 4 with immunosuppressive treatment

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**

O'Brien 2012 (*Continued*)

Random sequence generation (Trials)	High risk	-
Allocation concealment (Trials)	High risk	-
Blinding of participants and personnel (Trials)	High risk	-
Blinding of outcome assessment (Trials)	High risk	-
Selection of participants into the study (Prospective observational studies)	High risk	Small numbers lost to follow-up, but 10 were excluded because treatment was ongoing, therefore selection related to outcome present
Measurement of outcomes (Prospective observational studies)	Low risk	Outcomes were objective.
Incomplete outcome data / missing data (All studies)	Low risk	No missing data
Selective reporting (All studies)	Low risk	Reported all expected outcomes
Other bias	High risk	The proportion of participants receiving antibiotic treatment increased from 2005; there might be important differences between groups especially before this time. Study not really able to detect differences between treatment + surgery and surgery alone. 24/133 (18%) of participants had comorbidities that may have affected healing

O'Brien 2013b

Methods	Prospective observational study
Participants	Inclusion criteria: clinically + laboratory-confirmed BU; received antibiotics with or without surgery (exclusion criteria: none stated) Laboratory confirmation: any of (1) a culture of *Mycobacterium ulcerans* from the lesion, (2) PCR(+), or (3) histopathology showing a necrotic granulomatous ulcer with the presence of AFB Enrolled: 160 participants; 2 deaths, 2 lost to follow-up; 156 participants analysed Participant characteristics: 86 males, 55.1%; 13 participants (8.3%) < 15 years, 62 participants (39.7%) 15 to 59 years, 81 participants (51.9%) > 60 years Lesion types: ulcer 137 (87.8%), nodules 10 (6.4%), oedematous lesion 9 (5.8%) WHO classification: N/A
Interventions	Different oral antibiotic treatments. Participants received combinations of the following. • Rifampicin 147 (94.2%) • Ciprofloxacin 101 (64.7%)

	• Clarithromycin 48 (30.8%) • Ethambutol 11 (7.1%) • Amikacin 5 (3.2%) • Moxifloxacin 2 (1.5%) Drug dosages • Rifamipicin 10 mg/kg/day (up to a maximum of 600 mg/day) • Ciprofloxacin 500 mg twice daily • Clarithromycin 7.5 mg/kg/twice daily (up to maximum of 500 mg twice daily) • Moxifloxacin 400 mg daily • Amikacin 15 mg/kg/day Surgery: when indicated Follow-up: at least 12 months
Outcomes	1. Episodes of paradoxical reactions 2. Lesion site 3. Diagnosis and treatment 4. Healing of paradoxical reactions 5. Predictors of paradoxical reactions
Notes	Trial location: Australia Enrolment dates: 1 January 1998 to 31 December 2011 13 (8.3%) participants were complicated with diabetes mellitus and 11 (7.1%) with immune suppression (defined as current treatment with immunosuppressive medication (for example, prednisolone) or an active malignancy)

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (Trials)	High risk	-
Allocation concealment (Trials)	High risk	-
Blinding of participants and personnel (Trials)	High risk	-
Blinding of outcome assessment (Trials)	High risk	-
Selection of participants into the study (Prospective observational studies)	Low risk	Small number (4) not included as did not have 12 months follow-up or had died
Measurement of outcomes (Prospective observational studies)	Low risk	Paradoxical reaction clearly defined.
Incomplete outcome data / missing data (All studies)	Low risk	No missing data
Selective reporting (All studies)	Low risk	Reported all expected outcomes

O'Brien 2013b (*Continued*)

Other bias	Low risk	No other bias identified.

Phillips 2014a

Methods	Prospective observational study
Participants	Inclusion criteria: clinically + laboratory-confirmed BU; at least 5 years of age, presented with lesions ≤ 15 cm in diameter (exclusion criteria: tuberculosis or leprosy; renal or hepatic impairment, auditory problems; under treatment with antibiotics or herbal preparations; pregnancy) Laboratory confirmation: IS2404 dry-reagent-based PCR Enrolled: 82 patients screened for BU; 17 not meeting clinical and or epidemiological criteria for BU, 18 large category III lesions, 1 pregnancy, 3 were below 5 years; 43 for analysis Participant characteristics: 18 males, 42%; median age 15 (range, 5 to 70) Lesion types: ulcer 20 (47%), nodules 14 (32%), plaque 9 (21%) WHO category I: 27 (63%), category II: 12 (28%), category III: 4 (9%)
Interventions	Rifampicin (10 mg/kg/d) + streptomycin (15 mg/kg/d), 2 weeks followed by rifampicin (10 mg/kg/d) + clarithromycin (7.5 mg/kg/d), 6 weeks The treatment was administered under the direct observation of village health workers Surgery: when indicated; surgery and skin grafting was offered to participants whose lesion had enlarged during or after treatment by more than 150% of the initial size or had not healed by week 52 Follow-up: 52 weeks
Outcomes	1. Healing of the *Mycobacterium ulcerans* lesion without recurrence within 12 months of treatment commencement **(cure)** 2. Healing time 3. Recurrence 4. Adverse event (vestibulocochlear toxicity) 5. Paradoxical reactions
Notes	Trial location: Ghana Enrolment dates: July 2009 to July 2010

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (Trials)	High risk	-
Allocation concealment (Trials)	High risk	-
Blinding of participants and personnel (Trials)	High risk	-

Phillips 2014a (*Continued*)

Blinding of outcome assessment (Trials)	High risk	-
Selection of participants into the study (Prospective observational studies)	Low risk	Selection not related to intervention or outcome.
Measurement of outcomes (Prospective observational studies)	Low risk	Objective outcome measures
Incomplete outcome data / missing data (All studies)	Low risk	Only 2 (5%) participants were lost to follow-up.
Selective reporting (All studies)	Low risk	Reported all expected outcomes
Other bias	Low risk	No other bias identified.

Revill 1973

Methods	Randomized controlled trial
Participants	Inclusion criteria: clinically diagnosed BU (exclusion criteria: none stated) Laboratory confirmation: 22/105 (21%) participants had positive cultures for *Mycobacterium ulcerans*. Enrolled: 106 participants; 1 excluded; 105 participants for analysis Participant characteristics: sex N/A; age N/A Lesion types: ulcer 34/105 (33%) WHO category: N/A
Interventions	Participants were placed into 4 groups: 1. uncomplicated non-ulcerated lesions with immediate surgery withheld (Group A: 34 participants, 32.5%); 2. uncomplicated non-ulcerated lesions with immediate surgery (Group B: 16 participants, 15%); 3. complicated non-ulcerated lesions with immediate surgery (Group C: 21 participants, 20%); 4. ulcerated lesion with immediate surgery (Group D: 34 participants, 32.5%). They were randomized to the following groups. 1. Clofazimine (10 to 20 mg/kg/day) continued for at least 1 month after complete clinical healing (3 to 6 months) 2. Placebo capsule Surgery: when indicated Follow-up: participants were followed up every 2 weeks at a clinic in the trial area. Those who did not attend were visited at home. After the treatment period, participants were seen at approximately 3-monthly intervals. The follow-up period ranged from 17 to 40 months (median of 32 months)
Outcomes	1. Healing 2. Median healing time 3. Recurrence

Revill 1973 (*Continued*)

	Standarized outcome: possible cure	
Notes	Trial location: Uganda Enrolment dates: July 1968 to March 1970	
Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (Trials)	High risk	Only partial group was randomized.
Allocation concealment (Trials)	Low risk	Allocation concealed from both participant and doctor.
Blinding of participants and personnel (Trials)	Low risk	Placebo capsule was used, and both participant and doctor were blinded
Blinding of outcome assessment (Trials)	Unclear risk	No information about whether outcome assessors were blinded
Selection of participants into the study (Prospective observational studies)	Unclear risk	-
Measurement of outcomes (Prospective observational studies)	Unclear risk	-
Incomplete outcome data / missing data (All studies)	Low risk	Only 1 participant missing, and reason explained and unlikely to affect outcome
Selective reporting (All studies)	Low risk	Reported all expected outcomes
Other bias	Low risk	No other bias identified.

Sarfo 2010

Methods	Prospective observational study
Participants	Inclusion criteria: clinically + laboratory-confirmed BU (exclusion criteria: previous diagnosis of leprosy or TB; presence of renal or hepatic impairment or auditory problems; treatment with antibiotics or herbal preparations) Laboratory confirmation: diagnosis was confirmed by 1 or more methods. Swabs, punch biopsy specimen, or fine-needle aspirates were taken to test for the following 1. AFB 2. Culture for *Mycobacterium ulcerans* 3. PCR for IS2404 Enrolled: 171 participants; 6 participants with no diagnostic samples, 5 participants with negative laboratory results; 160 participants for analysis Participant characteristics: 66 males (41%), median 12 years (range, 1 to 75 years) Lesion types: ulcer 86 (53.7%), nodule 36 (22.5%), plaque 14 (8.8%), oedema 24 (15%)

Sarfo 2010 (*Continued*)

	WHO category I: 48 (30%), category II: 56 (35%), category III: 56 (35%)
Interventions	Rifampicin (10 mg/kg/d) + streptomycin (15 mg/kg/d) for 8 weeks Surgery: when indicated Follow-up period: 1 year after treatment completion
Outcomes	1. Healing of the *M ulcerans* lesion without recurrence within 12 months of treatment commencement **(cure)** 2. Healing time 3. Rate of healing of each measurable lesion 4. Recurrence until 12 months 5. Adverse effects 6. Paradoxical reactions
Notes	**Trial location:** Ghana **Enrolment dates:** September 2005 to December 2007

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (Trials)	High risk	-
Allocation concealment (Trials)	High risk	-
Blinding of participants and personnel (Trials)	High risk	-
Blinding of outcome assessment (Trials)	High risk	-
Selection of participants into the study (Prospective observational studies)	Low risk	Selection not related to intervention or outcome.
Measurement of outcomes (Prospective observational studies)	Low risk	Objective outcome measures
Incomplete outcome data / missing data (All studies)	Low risk	1 death and 1 lost to follow-up at 1 year
Selective reporting (All studies)	Low risk	Reported all expected outcomes
Other bias	Low risk	No other bias identified.

Abbreviations: AFB: acid-fast bacilli; BU: Buruli ulcer; IQR: interquartile range; N/A: not available; PCR: polymerase chain reaction; SD: standard deviation; TB: tuberculosis; WHO: World Health Organization; ZN: Ziehl-Neelsen.

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Addison 2015	Conference proceeding
Adjei 1998	Wrong study design
Adou 2009	Review
Adu 2011	Wrong study design
Adu 2015	Wrong study design
Aguiar 1997	Wrong study design
Alferink 2013	Wrong study design
Alffenaar 2010	Duplicate
Anonymous 2010	Review
Arens 2015	Conference proceeding
Azanmasso 2013	Wrong outcomes
Bamberger 2011	Review
Barogui 2009	Wrong study design
Barogui 2013	Wrong study design
Cornet 1992	Wrong study design
Cowan 2015	Wrong study design
Darie 1993	Wrong study design
de Bergeyck 1980	Wrong study design
Debacker 2005	Wrong study design
Friedman 2012	Wrong intervention
Gordon 2010	Wrong study design
Guerra 2008	Wrong study design

(*Continued*)

Josse 1994	Wrong study design
Kanga 2003	Wrong study design
Kibadi 2007	Wrong study design
Klis 2014a	Duplicate
Klis 2014b	Commentary
Klis 2014c	Duplicate
Klis 2014d	Commentary
Klis 2016	Wrong study design
Kotey 2011	Conference proceeding
Lunn 1965	Review
Marion 2015	Wrong study design
Milánkovits 2010	Commentary
Mou 2015	Wrong outcomes
Nienhuis 2012	Duplicate
O'Brien 2014	Wrong intervention
Oluwasanmi 1975	Wrong study design
Pfau 2015	Conference proceeding
Phanzu 2006	Wrong study design
Phanzu 2011	Wrong study design
Phillips 2004	Wrong intervention
Phillips 2014b	Commentary
Ruf 2011	Wrong study design
Ruf 2015	Wrong intervention

(*Continued*)

Saka 2013	Wrong study design
Schunk 2009	Wrong study design
Schütte 2009	Wrong setting
Stienstra 2012	Wrong study design
Teelken 2003	Wrong study design
van der Werf 1989	Wrong study design
Vignier 2014	Wrong study design
Vuagnat 2011	Wrong intervention
Yeboah-Manu 2013	Conference proceeding

Characteristics of ongoing studies *[ordered by study ID]*

NCT01432925

Trial name or title	Timing of surgical intervention in Buruli ulcer patients treated with antibiotics (Burulitime)
Methods	Randomized controlled trial (single-blind)
Participants	Inclusion criteria: aged 3 years and older, all stages of the BU disease with confirmation by direct microscopy following acid-fast staining or PCR Exclusion criteria: patients not on the standard treatment of 8 weeks of rifampicin and streptomycin for any reason, including non-compliant patients; treatment with macrolide or quinolone antibiotics, or antituberculous medication, or immunomodulatory drugs including corticosteroids within the previous 1 month; contraindication for general anaesthesia; pregnancy; osteomyelitis; lesion close to the eye; refusal to surgery at any point in the intended treatment; HIV positive; lack of willingness to give informed consent Estimated enrolment: 260
Interventions	1. Rifampicin (10 mg/kg/d) + streptomycin (15 mg/kg/d), 8 weeks plus surgery at week 8 2. Rifampicin (10 mg/kg/d) + streptomycin (15 mg/kg/d), 8 weeks plus surgery at week 14
Outcomes	Primary outcome 1. Healing without surgical intervention (time frame: 1 year) Secondary outcomes 1. Extent of surgery by measurement of lesional size 2. Functional limitations after the end of treatment and 1 year after the start of treatment 3. Duration of admission

NCT01432925 (*Continued*)

Starting date	September 2011 Anticipated end date: January 2017
Contact information	Ymkje Stienstra, MD PhD, University Medical Center Groningen
Notes	Trial location: Benin Registration number: NCT01432925

NCT01659437

Trial name or title	Randomized controlled trial comparing efficacy of 8 weeks treatment with clarithromycin and rifampicin versus streptomycin and rifampicin for Buruli ulcer (*Mycobacterium ulcerans* infection)
Methods	Randomized controlled trial (multicentre, open-label)
Participants	Inclusion criteria: aged 5 years and older, with a clinical diagnosis of BU disease (categories I and II, cross-sectional diameter ≤ 10 cm) as agreed by study site treatment team led by the lead clinicians Exclusion criteria: lesion sizes > 10 cm in cross-sectional diameter; children < 5 years, or < 20 kg body weight; pregnancy; previous treatment of Buruli ulcer, tuberculosis, or leprosy with at least 1 of the study drugs (rifampicin, streptomycin, clarithromycin); history of hypersensitivity to rifampicin and/or streptomycin and/or clarithromycin; previous treatment with macrolide or quinolone antibiotics, or antituberculosis medication, or immunomodulatory drugs including corticosteroids within 1 month; current treatment with any drugs likely to interact with the study medication; co-infection with HIV; history or having current clinical signs of ascites, jaundice, partial or complete deafness, myasthenia gravis, renal dysfunction (known or suspected), diabetes mellitus, and severe immune compromise (for example, immunosuppressive drugs after organ transplant), or evidence of (previous) tuberculosis, Buruli ulcer or leprosy, or terminal illness (for example, metastasized cancer); unable to take oral medication or having gastrointestinal disease likely to interfere with drug absorption; individuals with known or suspected bowel strictures who cannot tolerate macrolide antibiotics such as clarithromycin; mental condition likely to interfere with ability to comply with the study protocol Estimated enrolment: 415
Interventions	1. Rifampicin (10 mg/kg/d) + streptomycin (15 mg/kg/d), 8 weeks 2. Rifampicin (10 mg/kg/d) + clarithromycin (7.5 mg/kg/d), 8 weeks
Outcomes	Primary outcome 1. Healing without recurrence and without excision surgery (time frame: 12 months after start of treatment) Secondary outcomes 1. Recurrence rate within 12 months of treatment initiation 2. Number of recurrent lesions occurring after initial healing within 12 months of treatment initiation 3. Rate of treatment failure within 12 months of treatment initiation 4. Rate of paradoxical response within 12 months of treatment initiation 5. Proportion of participants with reduction in lesion surface area within 12 months of treatment initiation 6. Time taken for complete lesion healing within 12 months of treatment initiation 7. Proportion (%) of participants with complete healing without additional surgery or relapse 8. Interval between healing and recurrence

NCT01659437 (*Continued*)

	9. Proportion of each type of surgery within 12 months of treatment initiation 10. Time from treatment initiation to surgery if any 11. Proportion of participants with residual functional limitations 12. Treatment discontinuation and compliance rates 13. Incidence of all adverse effects within 12 months of treatment initiation
Starting date	December 2012 Anticipated end date: January 2018
Contact information	Tjip S van der Werf, Professor, University Medical Center Groningen (t.s.van.der.werf@umcg.nl)
Notes	Trial location: 1 centre in Benin and 4 centres in Ghana Registration number: NCT01659437

Abbreviations: BU: Buruli ulcer; PCR: polymerase chain reaction

DATA AND ANALYSES

Comparison 1. Rifampicin combined with streptomycin versus surgery alone

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Recurrence	1	21	Risk Ratio (M-H, Fixed, 95% CI)	0.12 [0.01, 2.51]

Comparison 2. Rifampicin combined with clarithromycin versus rifampicin combined with streptomycin in the consolidation phase

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Cure	1	151	Risk Ratio (M-H, Fixed, 95% CI)	0.94 [0.87, 1.03]
2 Recurrence at 12 months	1	151	Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]

ADDITIONAL TABLES

Table 1. Monotherapy for treating Buruli ulcer: description of studies and main results

Regimen	Study	Design	Comparisons	Surgery	Inclusion criteria (age, lesion)	Laboratory (Y/N)	N	Sex (M:F)	Age	Lesion types	Question	Outcome measure and time point (number analysed if different from N)	Results	Observation
CLF	Revill 1973	RCT 1.	2 groups: Rx at least until	When indicated	None	N	105	NR	NR	Ulcer: 34 (32%) Non-ul-	Does CLF reduce recurrence	Recurrence 1.	8/51 (15.7%)	No obvious effect

Table 1. Monotherapy for treating Buruli ulcer: description of studies and main results (*Continued*)

			1 month after complete clinical healing (3 to 6 months Placebo							cer: 71 (68%)	rates?	2.	10/54 (18.5%)	
											Is CLF effective?	Healed (n = 34)[a]	1. 5/13 (38%) 2. 6/21 (29%)	No obvious effect
											Does CLF shorten the healing time?	Median healing time (n = 25)[b]	1. 21 weeks (n = 8) 2. 14 weeks (n = 17)	No obvious effect
CLF	Lunn 1964	POS	1 group: Rx for 1 to 4 weeks followed by surgery	All	None	N	10	6:4	Mean 12.7 (5 to 25)	Ulcer: 10 (100%)	What is the healing rate for participants treated with CLF and surgery?	Healed	6/10 (60%)	Early study investigating the possible effect of treatment of BU with Rx. Healing rate with

Table 1. Monotherapy for treating Buruli ulcer: description of studies and main results (*Continued*)

														CLF plus surgery was 60%. The sample size is too small to draw any conclusion from this study
TMP/SMX	Fehr 1994	RCT	2 groups: 1. Rx until further excision became necessary (n = 6) 2. Placebo (n = 6)	When indicated	None	Y	12	5:7	1. Mean 18.3 (5 to 32) 2. Mean 20.8 (8 to 45)	Ulcer: 12 (100%)	Is TMP/SMX effective?	% change in ulcer size at study end[c,d] % covered by granulation tissue at study end	1. -10.9% (-26% to -6%) 2. 24.5% (-15% to 166%) 1. 92% (70% to 100%) 2. 57% (0 to 100%)	No obvious effect

[a] Healing was measured in 34 participants with non-ulcerated lesions who were withheld from immediate surgery.
[b] Healing time was measured in 25 participants with non-ulcerated lesions who were withheld from surgery and had small lesions (< 5 cm in diameter).
[c] 4 participants in group 1 and 2 participants in group 2 had surgery prior to intervention.
[d] Initial mean ulcer size: (1) 73.8 cm^2 (9 to 247), (2) 38.7 cm^2 (15 to 80).
Abbreviations: CLF, clofazimine; TMP/SMX, sulfamethoxazole/trimethoprim; Lab, laboratory confirmation; NR, not reported; POS, prospective observational study; RCT, randomized controlled trial; Rx, treatment.

Table 2. Rifampicin combined with streptomycin for treating Buruli ulcer: description of studies and main results

Regimen	Study	Design	Comparisons	Surgery	Inclusion (age, lesion)	Laboratory (Y/N)	N	Sex (M:F)	Age	Lesion types	Question	Outcome measure and time point (number analysed if different from N)	Results	Our observation
RFP and SM	Etuaful 2005	RCT	5 groups: Rx given for 2, 4, 8, 12 weeks prior to surgery versus surgery alone	All	≥ 15 years Non-ulcer Size < 10 cm	Y	21	7:14	NR	Nodule: 14 (67%) Plaque: 7 (33%)	Does RFP + SM before surgery reduce recurrence?	Recurrence, 12 months	Rx + surgery: 0/16 Surgery alone: 1/5	No obvious effect
											Does RFP + SM reduce lesion	Mean surface area reduction in	2 weeks, 5 participants: 29% 4 weeks, 3 participants: 52% 8 weeks, 5 par-	All tend to get smaller over time. No obvious effect of longer treatments

Table 2. Rifampicin combined with streptomycin for treating Buruli ulcer: description of studies and main results (*Continued*)

													tici-pants: 31% 12 weeks, 3 par-tici-pants: 41%	
RFP and SM	Kibadi 2010	POS	1 group: Rx for 12 weeks	All (at week 4)	3 to 75 years Ulcer Size > 10 cm	N	92	43:49	< 15 years: 38 (41%) ; 15 to 49 years: 43 (47%) ; ≥ 50 years: 11 (12%)	Ulcer: 92 (100%)	Is RFP + SM for 12 weeks with surgery at week 4 effec-tive?	Healed with Rx + surgery, 12 weeks	85/92 (92. 4%)[b]	Surgery plus 12 weeks of Rx asso-ciated with high heal-ing and low recur-rence at 24 months in large le-sions. Inde-pen-dent effect of antibi-otics not evalu-ated
												Recur-rence, 24 months	2/ 92 (2. 2%)[c]	
RFP and SM	Chauty 2007	POS	1 group: Rx for 8 weeks	When indi-cated (at week 4,	None	N	224	145: 79	< 15 years: 179 (58%) ; 15 to	Ulcer: 168 (75%) Non-ul-cer: 56	Does RFP + SM for 8 weeks work?	Healed with Rx ± surgery, after 8	206/ 206 (100%) [d]	Regi-men com-bined with surgery

Table 2. Rifampicin combined with streptomycin for treating Buruli ulcer: description of studies and main results (*Continued*)

				week 8)					49 years: 90 (29%) ; ≥ 50 years: 41 (13%)	(26%)		weeks (n = 206)[d]		as needed was associated with high healing rate after 8 weeks and low recurrence at 12 months. 48% of participants healed with Rx alone. 52% required surgery to heal
												Healed with Rx alone, after 8 weeks (n = 206)[d]	98/206 (48%)[e]	
												Underwent surgery (n = 206)[d]	108/206 (52%)[f,g]	
												Healed with Rx + surgery, after 8 weeks (n = 108)	108/108 (100%)	
												Recurrence, 12 months (n = 208)[h]	3/208 (1.4%)[i]	
RFP and SM	Sarfo 2010	POS	1 group: Rx for 8	When indicated (post-	None	Y	160	66:94	Median 12	Ulcer: 86 (54%)	Does RFP + SM for	Healed with	158/159 (99.	Regimen com-

Table 2. Rifampicin combined with streptomycin for treating Buruli ulcer: description of studies and main results (*Continued*)

			weeks	Rx; after week 8)					years (1 to 75)	Nodule: 36 (22%) Plaque: 14 (9%) Oedem: 24 (15%)	8 weeks work?	Rx ± surgery, 12 months (n = 159)[j]	3%)	bined with surgery as needed was associated with high healing rate and low recurrence at 12 months. 95% of participants healed with Rx alone. 5% required surgery to heal
												Healed with Rx alone, 12 months (n = 159)[j]	151/159 (95%)	
												Underwent surgery (n = 159)[j]	8/159 (5%)[k]	
												Healed with Rx + surgery, 12 months (n = 8)	7/8 (87.5%)[l]	
												Recurrence, 12 months (n = 158)[m]	0/158 (0%)	
RFP and SM	Adu 2013	POS	1 group: Rx for 8 weeks	When indicated (post-Rx; af-	None	Y	126	64:62	Mean 29.8 years (1	Ulcer: 116 (92%)	Does RFP + SM for 8	Healed with Rx	61/126 (48%)	About half (48%) healed

Table 2. Rifampicin combined with streptomycin for treating Buruli ulcer: description of studies and main results (*Continued*)

				ter week 8)					year 3 months to 98)	Papule: 1 (0.5%) Nodule: 2 (1.5%) Oedem: 4(3%) Osteo: 2 (1.5%) Contracure: 2 (1.5%)	weeks work?	alone, 8 weeks		with Rx alone. The other half underwent surgery including excision, skin grafting, and contracture release
RFP and SM	Agbenorku 2011	POS	1 group: Rx for 8 weeks	All (during or post-Rx)	None	Y	189	113: 76	NR	Ulcer: 145 (77%) Nodule: 38 (20%) Plaque: 6 (3%)	Does RFP + SM for 8 weeks with surgery work?	Healed with Rx + surgery, 2 years [n]	182/189 (96.3%)	Surgery plus 8 weeks of Rx was associated with high healing rate and low recurrence. Independent effect of Rx not evaluated

Table 2. Rifampicin combined with streptomycin for treating Buruli ulcer: description of studies and main results (*Continued*)

												Recurrence, 2 years	1/189 (0.5%)	
RFP and SM	Beissner 2015	POS	1 group: Rx for 8 weeks	When indicated (post-Rx; after week 8)	None	Y	129	60:69	Median 10 years (2 to 68)	Ulcer: 73 (57%) Nodule: 19 (15%) Plaque: 26 (20%) Oedema 11 (8%)	Does RFP + SM for 8 weeks work?	Healed with Rx ± surgery, > 6 months	109/129 (84.5%)[o]	Regimen combined with surgery as needed was associated with relatively high healing rate and no recurrence at minimum 6 months. 70% of participants healed with Rx alone. 27% of participants required surgery, of which 54% healed

Table 2. Rifampicin combined with streptomycin for treating Buruli ulcer: description of studies and main results (*Continued*)

												Healed with Rx alone, > 6 months	90/129 (69.8%)
												Underwent surgery, > 6 months	35/129 (27%)
												Healed with Rx + surgery, 6 months (n = 35)	19/35 (54%)
												Recurrence, > 6 months	None

[a] 16 participants who received Rx plus surgery were analysed. Participant characteristics for this group were: M:F = 6:10; age: NR; lesion types: nodule, 11 (69%), plaque, 5 (31%).
[b] PCR(+) group, 60/61 (98.4%); PCR(-) group, 25/30 (83.3%).
[c] 2/61(3.3%) among PCR(+) group.
[d] 17 lost to follow-up; 1 death.
[e] Ulcer size < 5 cm, 22/98 (22.5%); ulcer size 5 to 14 cm, 40/98 (41%); ulcer size >= 15 cm, 12/98 (12%); non-ulcer, 24/98 (24.5%).
[f] Ulcer size < 5 cm, 5/108 (4.6%); ulcer size 5 to 14 cm, 27/108 (25%); ulcer size >= 15 cm, 46/108 (42.6%); non-ulcer, 30/108 (27.8%).
[g] Decision to perform surgery was made by a treating physician: immediate upon enrolment, 4/108 (3.7%); 4-week assessment, 83/108 (76.9%); 8-week assessment, 21/108 (19.4%).
[h] 208 participants were retrieved for 1-year follow-up.
[i] 2 among the Rx-only group; 1 among the Rx + surgery group.
[j] 1 death.
[k] 2 participants with nodules, 1 participant with plaque, 5 participants with ulcerated oedematous lesions, and 2 participants with large ulcers were offered surgical intervention after 8 weeks of Rx; 8 accepted surgery.
[l] 1 participant evaluated as treatment failure in this review although successful treatment at 1 year follow-up. The participant received additional 4 weeks of RFP + SM (a total of 12 weeks) with breaking down of skin grafting with culture positive during course of treatment.
[m] 158 participants were retrieved for 1-year follow-up after treatment completion.

[n] Debridement and skin grafting included as surgery: 38 participants (20.1%) with nodules or plaque excised, 151 participants with ulcers (79.9%) had debridement and skin grafting.
[o] 5 participants (3.9%) had secondary lesions, and 15 participants (11.6%) had functional limitations.
Abbreviations: Lab, laboratory confirmation; NR, not reported; Osteo, osteomyelitis; POS, prospective observational study; RCT, randomized controlled trial; RFP, rifampicin; Rx, treatment; SM, streptomycin.

Table 3. Rifampicin combined with clarithromycin for treating Buruli ulcer: description of studies and main results

Regimen	Study	Design	Comparisons	Surgery	Inclusion (age, lesion)	Laboratory (Y/N)	N	Sex (M:F)	Age	Lesion types	Question	Outcome measure and time point (number if different from N)	Results	Our observation
RFP and CAM	Chauty 2011	POS	1 group: Rx given for 8 weeks	When indicated	≥ 5 years Size ≤ 10 cm	Y	30	18:12	NR	Ulcer: 21 (70%) Non-ulcer: 9 (30%)	Does 8 weeks of RFP + CAM work?	Healed with Rx ± surgery, 12 months	30/30 (100%)	Regimen combined with surgery as needed was associated with high healing rate at 12 months and no recurrence at 18 months

Table 3. Rifampicin combined with clarithromycin for treating Buruli ulcer: description of studies and main results (*Continued*)

													in participants with small lesions. 50% of participants healed with Rx alone. 50% required surgery to heal
											Healed with Rx alone, 12 months	15/30 (50%) [a]	
											Underwent surgery, 12 months	15/30 (50%)	
											Healed with Rx + surgery, 12 months (n = 15)	15/15 (100%) [b]	
											Recurrence, 18 months	None	
RFP and CAM	O'Brien 2012	POS	1 group: Rx, duration depending upon physi-	All	None	Y	21	NR	NR	NR	Does RFP + CAM plus surgery work?	Healed with Rx + surgery, 12 months	21/21 (100%)	Surgery plus Rx was associated with high

Table 3. Rifampicin combined with clarithromycin for treating Buruli ulcer: description of studies and main results (*Continued*)

			cian's decision										healing rate and no recurrence at 12 months. Independent effect of antibiotics not evaluated. Duration of Rx varied
											Recurrence , 12 months	None	
RFP and SM *plus* RFP and CAM	BURULICO Study 2010	RCT	2 groups: 1. RFP + SM for 4 weeks (4RS) followed by RFP + CAM for 4 weeks (4RC) 2. RS for 8 weeks (8RS)	When indicated	≥ 5 years Size ≤ 10 cm	Y	151	46: 105	1. Median 12 years (IQR 9 to 22) 2. Median 12 years (IQR 8 to 18)	Ulcer: 59 (39%) Non-ulcer: 92 (61%)	Can RFP + CAM substitute for RFP + SM?	Healed with Rx alone or Rx + skin grafting, 12 months	1. 68/75 (91%) 2. 73/76 (96%) [c,d]	4RS + 4RC was as effective as 8RS in participants with small lesions. Both regimens were associated with high heal-

Table 3. Rifampicin combined with clarithromycin for treating Buruli ulcer: description of studies and main results (*Continued*)

													ing rate and no recurrence at 12 months
											Difference in healing time	None[e]	
											Recurrence, 12 months	None	
RFP and SM *plus* RFP and CAM	Phillips 2014a	POS	1 group: RFP + SM for 2 weeks (2RS) followed by RFP + CAM for 6 weeks (6RC)	When indicated	≥ 5 years Size ≤ 15 cm	Y	43	18:25	Median 14 years (5 to 70)	Ulcer: 20 (47%) Nodules: 14 (32%) Plaque: 9 (21%)	Can RFP + CAM substitute for RFP + SM?	Healed with Rx ± surgery, 12 months (n = 41)[f]	41/41 (100%)	2RS + 6RC combined with surgery as needed was associated with high healing and low recurrence at 12 months in participants

Table 3. Rifampicin combined with clarithromycin for treating Buruli ulcer: description of studies and main results (*Continued*)

														with small lesions. 98% of participants healed with Rx alone. 2RS + 6RC may substitute for 8RS, but no definitive conclusion could be made as they were not compared
												Healed with Rx alone, 12 months (n = 41)[f]	40/41 (98%)	
												Underwent surgery (n = 41)[f]	1/41 (2.4%)	
												Healed with Rx + surgery, 12 months (n = 1)	1/1 (100%)[g]	
												Recurrence, 12 months (n = 41)[f]	None	

[a] 8/10 (80%) with ulcerative-WHO category I lesion; 5/11 (45%) with ulcerative-WHO category II lesion; 2/3 (66%) with non-ulcerative-WHO category I lesion; 0/6 (0%) with non-ulcerative-WHO category II lesion.

[b] 11 (37%) with limited surgery; 4 (13%) with excision and skin grafting.

[c] Skin grafting without excision: (1) 1/75 (1.3%); (2) 4/76 (5.3%).

[d] Odds ratio 2.49, 95% confidence interval 0.66 to infinity; P = 0.16, 1-sided Fisher's exact test.

[e] Group proportional hazard model: P = 0.26; 99% confidence interval 0.22 to 0.29; generalized Wilcoxon-Mann-Whitney test: P = 0.60; 99% confidence interval 0.56 to 0.64.
[f] 2 lost to follow-up.
[g] Skin grafting at week 32.
Abbreviations: CAM, clarithromycin; IQR, interquartile range; Lab, laboratory confirmation; NR, not reported; POS, prospective observational study; RCT, randomized controlled trial; RFP, rifampicin; Rx, treatment; SM, streptomycin.

Table 4. Novel combination regimens for treating Buruli ulcer: description of studies and main results

Regimen	Study	Design	Comparison(s)	Surgery	Inclusion (age, lesion)	Laboratory (Y/N)	N	Sex (M:F)	Age	Lesion types	Question	Outcome measure and time point (number if different from N)	Results	Our observation
RFP and DDS	Espey 2002	RCT	2 groups: 1. Rx for 8 weeks (n = 15) 2. Placebo (n = 15)	None	> 4 years Ulcer	N	30	18:12	NR	Ulcer: 30 (100%)	Is RFP + DDS effective?	Improved after 2 months (n = 28)[a]	1. 82% (23/28 points) 2. 75% (21/28 points)[b]	Unable to assess the effect due to incongruent characteristics at baseline, however there seems to be no obvious effect
												Change in	14.0 cm^2	

Table 4. Novel combination regimens for treating Buruli ulcer: description of studies and main results (*Continued*)

												ulcer size after 2 months 2.	decrease (range, 3.8 to -159.0) 2.5 cm^2 decrease (range, 78.0 to -35.0)[c]	
RFP and either CIPRO, CAM, or MOX	Friedman 2016	POS	3 groups: 1. RFP + CIPRO (n = 80) 2. RFP + CAM (n = 50) 3. RFP + MOX (n = 2)	Limited surgical debridement when indicated[d] (extensive surgical excisions excluded)	None	Y	132	75:57	Median 49 years (1 to 95)	Ulcer: 110 (83.3%) Nodule: 9 (6.8%) Oedema 10 (7.6%) Plaque: 3 (2.3%)	Does RFP-based all-oral Rx regimen work?	Healed with Rx ± limited surgical debridement, 12 months	131/132 (99%)[e]	RFP-based all-oral regimens combined with limited surgical debridement as needed were associated with high healing rate and no

Table 4. Novel combination regimens for treating Buruli ulcer: description of studies and main results (*Continued*)

														recurrence at 12 months. 77% of participants healed with Rx alone. Some lesions may need less than 8 weeks of Rx to achieve healing. These were less severe patients, as patients who required extensive surgical excision were

Table 4. Novel combination regimens for treating Buruli ulcer: description of studies and main results (*Continued*)

														excluded. No independent results were given for different regimens
												Healed with Rx alone, 12 months	101/132 (76.5%)	
												Median duration of therapy	56 days (IQR 24 to 96 days)	
												Duration of therapy: < 8 weeks	22/132 (16.7%)	
												Recurrence, 12 months	None	
Combination of RFP, CIPROCAM, ETB, MOX,	O'Brien 2012	POS	2 groups: 1. All-oral Rx (8 differ-	All	None	Y	133[f]	67:66	Median 62 years (3 to 94)	Ulcer: 106 (87%) Nodules: 9 (7%)	Is all-oral Rx treatment plus	Healed, 12 months[g]	90/90 (100%) 2. 33/47 (70%)	The tested all-oral regimen

Table 4. Novel combination regimens for treating Buruli ulcer: description of studies and main results (*Continued*)

AMK			ent regimens: see below) + surgery 2. Surgery alone Regimens: 1. RFP + CIPRO (n = 55) 2. RFP + CAM (n = 21) 3. RFP + CAM + ETB (n = 5) 4. CIPRO + CAM (n = 4) 5. RFP + MOX (n = 2) 6. CAM + ETB (n = 1) 7. RFP +							Oedem 7 (6%)[f]	surgery superior to just surgery:		[h]	plus surgery was associated with 100% healing and no recurrence at 12 months. 30% of participants who only had surgery had recurrence. Individual effect of Rx not evaluated

Table 4. Novel combination regimens for treating Buruli ulcer: description of studies and main results (*Continued*)

			ETB + AMK (n = 1) 8. CAM (n = 1)										
											Recurrence, 12 months[g]	1. 0/90 (0%) 2. 14/47 (30%)[i]	

[a] Judged by 2 Buruli ulcer specialists using photographs taken at enrolment and at 2 months. Photographs were available for 14 participants from each group; each evaluator's score was counted as 1 point.
[b] P = 0.51.
[c] P = 0.02; however, the initial median ulcer size was bigger in the treatment group than in the placebo group (26.2 cm^2 (0.25 to 280) versus 4.8 cm^2 (0.25 to 57.5), P = 0.04).
[d] Limited surgical debridement was defined as curettage of the lesion or a minor excision to remove excess granulation tissue and to debride ulcer margins, with or without the use of a split skin graft.
[e] Results not available for each individual regimen.
[f] Clinical type of lesion was recorded in 122/133 participants (92%).
[g] 137 lesions were analysed.
[h] P < 0.001.
[i] Median time of recurrence: 90 days (range, 14 to 300 days).
Abbreviations: AMK, amikacin; DDS, dapsone; CAM, clarithromycin; CIPRO, ciprofloxacin; ETB, ethambutol; IQR, interquartile range; Lab, laboratory confirmation; MOX, moxifloxacin; NR, not reported; POS, prospective observational study; RCT, randomized controlled trial; RFP, rifampicin; Rx, treatment; SM, streptomycin.

Table 5. Paradoxical reactions in Buruli ulcer: description of studies and main results

Regimen	Study	Design	Comparisons	Surgery	N	Sex (M:F)	Age	Lesion types	Incidence of PR	Onset time of PR	Our observation
RFP and SM	Sarfo 2010	POS	1 group: RFP + SM for 8 weeks (8RS)	When indicated (post-Rx)	160	66:94	Median 12 years (1 to 75)	Ulcer: 86 (54%) Nodule: 36 (22%) Plaque: 14 (9%) Oedema: 24 (15%)	3/159 (1.9%)[a]	At weeks 4, 6, 12	Approximately 1 in 20 participants treated with 8RS developed PR.

Table 5. Paradoxical reactions in Buruli ulcer: description of studies and main results (*Continued*)

											2 cases occurred during Rx and 1 case occurred post-Rx
RFP and SM or RFP and SM *plus* RFP and CAM	Barogui 2016	POS	1 group: 1. RFP + SM for 8 weeks (n = 166) 2. RFP + SM for 4 weeks followed by RFP + CAM for 4 weeks (n = 75)	When indicated	241	88:153	Mean (SD) 16 (13) years	Ulcer: 108 (45%) Nodule: 32 (13%) Plaque: 56 (23%) Oedema: 11 (5%) Mixed: 34 (14%)	52/241 (22%)[b]	Between week 8 and 12	Approximately 1 in 5 participants treated with Rx developed PR between week 8 and 12
RFP and SM *plus* RFP and CAM	Phillips 2014a	POS	1 group: RFP + SM for 2 weeks (2RS) followed by RFP + CAM for 6 weeks (6RC)	When indicated	43	18:25	Median 14 years (5 to 70)	Ulcer: 20 (47%) Nodules: 14 (32%) Plaque: 9 (21%)	4/41 (9.3%)[c]	Median 12 weeks (range, 4 to 32 weeks)	Approximately 1 in 10 participants treated with 2RS + 6RC developed PR at median 12 weeks after start of treatment
Combination of RFP, CIPRO, CAM, ETB, AMK, MOX	O'Brien 2012	POS	8 groups: Weeks of 1. RFP + CIPRO (n = 55)	All	90	NR	NR	NR	8/90 (8.9%)[d]	Median 48 days (range, 14 to 85 days)	Approximately 1 in 10 participants treated with differ-

Table 5. Paradoxical reactions in Buruli ulcer: description of studies and main results (*Continued*)

			2. RFP + CAM (n = 21) 3. RFP + CAM + ETB (n = 5) 4. CIPRO + CAM (n = 4) 5. RFP + MOX (n = 2) 6. CAM + ETB (n = 1) 7. RFP + ETB + AMK (n = 1) 8. CAM (n = 1)								ent regimens of Rx developed PR at median 8 weeks after start of treatment
Combination of RFP, CAM, ETB, AMK, MOX	O'Brien 2013b	POS	1 group: received Rx	When indicated	156	86:70	< 15 years: 13 (8%); 15 to 60 years: 62 (40%); > 60 years: 81 (52%)	Ulcer: 137 (87.8%) Nodules: 10 (6.4%) Oedema: 9 (5.8%)	32/156 (21%)[e]	Median 39 days (IQR 20 to 73 days)	Approximately 1 in 5 participants treated with different regimens of Rx developed PR at 5.6 weeks after start of treatment
RFP and either CIPRO, CAM,	Friedman 2016	POS	3 groups: Weeks of	Limited surgical de-	132	75:57	Median 49 years (1 to 95)	Ulcer: 110 (83.3%)	34/132 (26%)[d]	Median 48 days (IQR	Approximately 1 in 4 par-

Table 5. Paradoxical reactions in Buruli ulcer: description of studies and main results (*Continued*)

or MOX			1. RFP + CIPRO (n = 80) 2. RFP + CAM (n = 50) 3. RFP + MOX (n = 2)	bride-ment when in-dicated				Nodule: 9 (6.8%) Oedema: 10 (7. 6%) Plaque: 3 (2.3%)		29 to 69 days)	ticipants treated with dif-fer-ent regi-mens of Rx devel-oped PR at median 8 weeks after start of treat-ment

[a] One death.
[b] 37/166 (22%) received RFP + CIPRO; 15/75 (20%) received RFP + CAM.
[c] 2 participants lost to follow-up.
[d] Results not available for each individual regimen.
[e] Predictors of paradoxical reactions (multivariable analysis): age ≥ 60 years (risk ratio (RR) 2.84, 95% confidence interval (CI) 1.12 to 7.17; P = 0.03), oedematous lesion (RR 3.44, 95% CI 1.11 to 10.70; P = 0.03), use of amikacin in the initial Rx regimen (RR 6.33, 95% CI 2.09 to 19.18; P < 0.01).
Abbreviations: AMK, amikacin; CAM, clarithromycin; CIPRO, ciprofloxacin; ETB, ethambutol; IQR, interquartile range; Lab, laboratory confirmation; MOX, moxifloxacin; NR, not reported; POS, prospective observational study; PR, paradoxical reactions; RCT, randomized controlled trial; RFP, rifampicin; Rx, treatment; SD, standard deviation; SM, streptomycin.

CONTRIBUTIONS OF AUTHORS

Rie Roselyne Yotsu conceived the review question, extracted and analysed data, and co-ordinated and drafted the protocol and review. Marty Richardson extracted and analysed data, provided statistical advice, and edited the protocol and review. Norihisa Ishii supervised the clinical content and approved the final version prior to submission.

DECLARATIONS OF INTEREST

Rie Roselyne Yotsu acts as a WHO consultant for leprosy and also has financial support for an epidemiological study on Buruli ulcer in Cote d'Ivoire and in Ghana. However, there are no financial relationships with any organizations that might have an interest in the submitted work in the previous three years; and no other relationships or activities that could appear to have influenced the submitted work.

Marty Richardson has no known conflicts of interest.

Norihisa Ishii has no known conflicts of interest.

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DIFFERENCES BETWEEN PROTOCOL AND REVIEW

We did not include the planned methods for conducting meta-analyses in the review due to the small number of included studies and their heterogeneity.

We added paradoxical reactions to the Secondary outcomes.

We revised the 'Risk of bias' assessment. The method stated in the protocol was only applicable to randomized controlled trials and not to prospective observational studies.

We added the following search terms: 'Buruli and skin', 'mycobacterial skin ulcer*', and 'mycobacterium skin ulcer*'.