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Faraj Fajloun

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THESE DE DOCTORAT

DE

L'UNIVERSITÉ LIBANAISE

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Spécialité : *Microbiologie Moléculaire*

Unité de recherche : Laboratoire d'Innovation Thérapeutique (LIT)

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LA COMUE ANGERS – LE MANS

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Par

Faraj FAJLOUN

« **Maladies tropicales négligées à manifestations cutanées : vers un développement de nouvelles stratégies préventives, de diagnostic et de traitement** »

Thèse présentée et soutenue au Liban, le 13 septembre 2023

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LISTE DES PUBLICATIONS

Article 1. Fajloun F*, Ganlonon L*, Gnimavo RS, Sodjinou E, Habib A, Claco E, Agoundoté I, Adeye A, Catraye P, Al-Bayssari C, Moussa EH, Robbe-Saule M, Houezo JG, Kpoton GG, Ayélo AG, Gomez B, Johnson RC, Marsollier L, Marion E, Kempf M. An overview of 10 years of activity of a molecular laboratory Buruli ulcer diagnosis at a field hospital in Benin. J Clin Microbiol. 2023 May 22:e0027423. doi: 10.1128/jcm.00274-23. (IF : 11.67)

* Contributed equally to the work

Article 2: Gnimavo RS*, Fajloun F*, Al-Bayssari C, Sodjinou E, Habib A, Ganlonon L, Claco E, Agoundoté I, Houngbo OA, Anagonou EG, Biaoou CAO, Ayélo AG, Houezo JG, Boccarossa A, Moussa EH, Gomez B, Gine A, Sopoh GE, Marion E, Johnson RC, Kempf M. Importance of consultations using mobile teams in the screening and treatment of neglected tropical skin diseases in Benin. PLoS Negl Trop Dis. 2023 May 12;17(5):e0011314. doi: 10.1371/journal.pntd.0011314. (IF : 4.78)

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Article 3. Azour A, Al-Bayssari C, Dagher TN, Fajloun F, Fajloun M, Rolain JM. Clonal Dissemination of Plasmid-Mediated Carbapenem and Colistin Resistance in Refugees Living in Overcrowded Camps in North Lebanon. Antibiotics (Basel). 2021 Dec 2;10(12):1478. doi: 10.3390/antibiotics10121478. (IF : 5.22)

Communication orale lors de la journée scientifique de l'école doctorale de Biologie Santé-Angers, organisée par le Collège Doctoral Pays de la Loire-Ecole doctorale de Biologie Santé (10 min communication + 5 min questions)

Titre : « An overview of 10 years of activity of a molecular laboratory Buruli ulcer diagnosis at a field hospital in Benin ».

LISTE DES ABRÉVIATIONS

Liste des abréviations en anglais :

| | |
|-------------|-----------------------------------|
| BU : | Buruli ulcer |
| BU-LABNET : | Buruli Ulcer LABORatory NETwork |
| ELISA : | Enzyme-Linked ImmunoSorbent Assay |
| FNA : | Fine Needle Aspiration |
| NTDs : | Neglected Tropical Diseases |
| PCR : | Polymerase Chain Reaction |
| WASH : | WATER, Sanitation, Hygiene |
| WHO : | World Health Organization |
| WT : | Wild Type |

Liste des abréviations en français :

| | |
|----------|---|
| CC : | Contrôle Conjugué |
| CDTLUB : | Centre de Dépistage et de Traitement de la Lèpre et de l'Ulcère de Buruli |
| CDTUB : | Centre de Dépistage et de Traitement de l'Ulcère de Buruli |
| CTAL : | Centre de Traitement Anti-Lépreux |
| DHPS : | Dihydroptéroate Synthétase |
| LIT : | Laboratoire d'Innovation Thérapeutique |
| MB : | Multi-Bacillaire |
| MTN : | Maladie Tropicale Négligée |
| MTN-C : | Maladie Tropicale Négligées à manifestations Cutanées |
| MUT : | Mutant |
| OMS : | Organisation Mondiale de la Santé |
| PB : | Pauci-Bacillaire |
| PNLLUB : | Programme National de Lutte contre la Lèpre et l'Ulcère de Buruli |
| PNLMT : | Programme National de Lutte contre les Maladies Transmissibles |
| PNLUB : | Programme National de Lutte contre l'Ulcère de Buruli |
| UB : | Ulcère de Buruli |
| UC : | Universal Control |
| UG : | Unité Génome |

INTRODUCTION GÉNÉRALE

Les maladies tropicales négligées (MTN) constituent un groupe de 20 maladies infectieuses prévalentes dues à des bactéries, des protozoaires, des helminthes et des champignons. Elles sont principalement répandues dans les pays sous-développés, au climat tropical ou subtropical humide, notamment dans les zones rurales isolées loin des centres de santé, dans les zones de conflit et les zones dépourvues de l'eau propre, où les gens vivent dans de mauvaises conditions d'hygiène, à proximité des vecteurs de transmission (1). Les MTN affectent plus d'un milliard de personnes dans le monde et coûtent des milliards de dollars en termes de coûts de soins de santé et de perte de productivité. Cet énorme fardeau financier pèse lourd sur les économies des pays endémiques, fournissant un argument économique solide pour investir dans la prévention, le diagnostic et le traitement précoce de ces maladies (2).

Les maladies tropicales négligées à manifestations cutanées (MTN-C) constituent un sous-groupe des MTN, une famille constituée de neuf maladies qui présentent des manifestations cutanées, soit comme symptôme principal, soit comme caractéristique clinique associée (3). Ces maladies sont l'ulcère de Buruli, la lèpre, le pian, la gale, la filariose lymphatique, le mycétome, la leishmaniose cutanée, la leishmaniose dermique post-Kala-azar et l'onchocercose (4). Le pronostic vital peut être défavorable en l'absence de traitement approprié. Ces maladies peuvent entraîner chez des patients des déformations cutanées visibles voire des handicaps physiques avec des incapacités permanentes. Les victimes des MTN-C sont soumises à une stigmatisation sociale et une discrimination tout au long de leur vie. Dans certains pays, les personnes affectées sont rejetées par leur communauté, ce qui entraîne des dommages psychologiques importants (3,5). Enfin, ces maladies altèrent la qualité de vie de ces populations mal desservies (6).

Ces neuf MTN-C peuvent être traitées par des traitements qui sont bien définis par l'Organisation Mondiale de la Santé (OMS). Cependant, il est encore souvent difficile de mettre en place les outils nécessaires à leur diagnostic dans des pays à faible revenu. Le problème d'accès aux centres de soins dans les régions rurales aggrave également la situation (4). S'ajoute à cela un investissement dans la recherche qui n'est pas toujours à la hauteur des attentes (7).

Les problèmes majeurs liés aux MTN ont depuis de nombreuses années motivé l'OMS à mettre en place des stratégies pour les combattre. Les actions de lutte de l'OMS ont

débuté au début des années 2000 (8) et une première feuille de route qui a mis en place des stratégies de lutte visant à contrôler, éliminer ou éradiquer chacune des 17 MTN dans les pays endémiques du monde été publiée en 2012 (9). Cette feuille de route a eu un énorme écho auprès de la plupart des pays et de nombreux succès ont été observés. En 2020, la gestion et la prise en charge de la plupart des MTN avait bien avancé. Cependant, la lutte contre ces maladies se poursuit et en 2021, l'OMS a publié une deuxième feuille de route pour évaluer l'impact global de nouvelles actions dont les résultats sont attendus en 2030 (10).

S'agissant des MTN-C, l'OMS recommande depuis 2013 des stratégies de lutte intégrées qui ciblent en même temps ces maladies, étant donné qu'elles sont co-endémiques et qu'elles partagent des signes cliniques similaires (11). En 2022, l'OMS a d'ailleurs publié le premier rapport de lutte intégrée contre les MTN-C (12).

Les MTN-C sont depuis longtemps endémiques dans les pays d'Afrique de l'Ouest, en particulier au Nigeria, au Bénin, au Ghana, en Côte d'Ivoire, au Burkina Faso et au Togo (13). L'Assemblée mondiale de la santé, qui s'est tenue en mai 2013, et le Comité régional de l'OMS pour l'Afrique, qui a eu lieu en septembre de la même année, ont tous les deux préconisé la création de programmes de prise en charge des MTN, dont les MTN-C (14,15). L'année suivante, la Région africaine de l'OMS a publié une stratégie régionale pour les MTN dans laquelle l'une des cibles identifiées était l'élimination de la lèpre, le contrôle de l'ulcère de Buruli et l'éradication du pian. Afin d'atteindre cet objectif, la Région africaine de l'OMS a demandé la mise en œuvre de plans et de programmes intégrés de lutte au niveau des pays où ces maladies étaient endémiques. Ainsi, des progrès encourageants ont été observés (16).

Le Bénin, un pays d'Afrique de l'Ouest, est endémique pour plusieurs MTN-C surtout pour l'ulcère de Buruli et la lèpre (17). Comme dans d'autres pays d'Afrique de l'Ouest, le Bénin a mis en place un système de santé spécifique pour lutter contre les MTN-C. Ce système a fourni des efforts importants au fil des années pour combattre ces maladies en appliquant les recommandations de l'OMS. Il est formé par des programmes nationaux de lutte et par des structures de dépistage et de prise en charge des patients localisées dans les départements endémiques du pays. Ainsi, des Centres de Dépistage et de Traitement de l'Ulçère de Buruli (CDTUB) ont vu le jour. Ce sont des structures installées au sud du pays, où l'ulcère de Buruli est endémique. Ces structures ont ensuite évolué avec les recommandations de l'OMS et ont intégré la prise en charge de la lèpre. Ces centres ont par

la suite été appelés Centres de Dépistage et de Traitement de la Lèpre et de l'Ulcère de Buruli (CDTLUB) (18).

L'équipe de recherche ATOMycA (INSERM CNRS INCIT UMR 1302/EMR6001), dirigée par le Dr Estelle Marion à Angers, a depuis de nombreuses années, établi des collaborations avec le CDTLUB situé à Pobè, dans le département du Plateau, au sud du Bénin. Dans ce cadre, l'équipe a mis en place le diagnostic par PCR de l'ulcère de Buruli au laboratoire de Pobè en 2012. L'équipe ATOMycA a également développé des liens très forts avec les équipes médicales de ce centre qui réalisent des consultations foraines dans plusieurs régions du pays pour le dépistage intégré des MTN-C. Les médecins sur le terrain nous ont fait état d'un manque d'outils pour le diagnostic de ces infections.

Objectif de la thèse

Ce travail de thèse s'est développé dans le cadre d'un projet collaboratif mené entre le laboratoire de recherche ATOMycA INSERM CNRS INCIT UMR 1302/EMR6001 à l'Université et CHU d'Angers (France) et le Laboratoire d'Innovation Thérapeutique (LIT) de l'Université Libanaise (Liban). L'objectif global a été de valoriser le travail de terrain de l'équipe médicale du CDTLUB de Pobè pour le dépistage intégré des MTN-C et de participer à la mise en place d'outils permettant le diagnostic de certaines de ces maladies.

Vue globale des différentes parties de la thèse

La **première partie** de la thèse, intitulée « Maladies tropicales négligées à manifestations cutanées : contexte et prise en charge », constitue la partie bibliographique de la thèse. Elle est présentée sous forme de manuscrit, en cours de rédaction. La revue est intitulée « Intervention strategies for the management of tropical skin diseases: the context in Bénin ».

La **deuxième partie** de la thèse, est intitulée « Focus sur l'ulcère de Buruli et les autres maladies tropicales négligées endémiques au Bénin ». Elle est consacrée à une valorisation des travaux de l'équipe des médecins, des infirmières et des techniciens de laboratoire du CDTLUB, elle est constituée de 2 articles publiés :

Article 1 : « An overview of 10 years of activity of a molecular laboratory for Buruli ulcer diagnosis at a field hospital in Benin ». Ce travail présente les résultats de 10 ans de diagnostic microbiologique de l'ulcère de Buruli par le laboratoire de biologie moléculaire du CDTLUB installé par notre équipe de recherche en 2012. Il présente également l'évolution de ce laboratoire au fil des années et son investissement dans le diagnostic moléculaire d'autres MTN-C endémiques au Bénin.

L'article a été publié dans le journal *Journal of Clinical Microbiology*.

Article 2 : « Importance of consultations using mobile teams in the screening and treatment of neglected tropical skin diseases in Benin ». Ce travail montre l'importance des consultations mobiles, mises en place par l'équipe des médecins du CDTLUB, dans le but de réaliser des dépistages intégrés des MTN-C endémiques dans des zones rurales à distance de centres de santé.

L'article a été publié dans le journal *PLoS Neglected Tropical Diseases*.

La **troisième partie** de la thèse est intitulée « Mise en place du kit GenoType LepraeDR à Pobè au Bénin, pour la détection de la résistance aux antilépreux chez *Mycobacterium leprae* ». Elle est consacrée à la mise en place, au CDTLUB de Pobè, d'une technique d'identification de la résistance aux antilépreux chez *M. leprae*, dans le but d'optimiser le traitement des patients.

Le kit GenoType LepraeDR a dans un premier temps été testé au laboratoire de bactériologie du CHU d'Angers. Une procédure complète a ensuite été rédigée pour faciliter la mise en place du test au CDTLUB de Pobè.

Une discussion générale, suivie d'une conclusion viendront ensuite compléter ce manuscrit de thèse.

**Partie 1 : Maladies tropicales négligées
à manifestations cutanées : contexte et
prise en charge**

Avant-propos

La co-endémicité de certaines MTN-C ainsi que le fort degré de similitude des signes cliniques qu'elles possèdent, rendent leur diagnostic clinique souvent difficile. Afin d'aider les personnels de santé dans la reconnaissance et dans la prise en charge de ces maladies, l'OMS a publié en 2018 un guide de formation présentant les signes cliniques, les méthodes de diagnostic clinique et de confirmation biologique (4).

Parallèlement, afin de combattre les MTN-C, l'OMS a mis en place différentes stratégies. Un premier plan a ainsi vu le jour en 2007 (8) et en 2012, l'OMS a publié sa première feuille de route pour la lutte contre 17 MTN dont 7 MTN-C (9). Ces engagements ont permis à 42 pays d'éliminer au moins une des MTN et à 600 millions de personnes de guérir ou d'éviter l'infection (10). Cependant, des objectifs n'ont pas totalement été atteints et le fardeau des MTN est resté important malgré les progrès significatifs réalisés. En 2017, l'OMS a ajouté 3 maladies à la liste des MTN dont 2 présentant des manifestations cutanées. Une deuxième feuille de route a été publiée en 2021 dans le but de faire le point sur les stratégies mises en place dans la première feuille de route et de proposer un nouveau plan de lutte contre les 20 MTN (10). Dans un rapport complémentaire publié en 2022, l'OMS a ciblé les MTN-C par des stratégies intégrées (12).

Au Bénin, des efforts importants ont été déployés au fil des années pour appliquer toutes les recommandations de l'OMS annoncées par les rapports et les feuilles de route. Des programmes et des structures de lutte contre les MTN-C ont ainsi vu le jour. Cependant, le pays s'est heurté à plusieurs difficultés pour développer ces stratégies sur le terrain.

Cette revue bibliographique s'intéresse aux MTN-C, à leur prise en charge et aux problématiques rencontrées par les acteurs sur le terrain Béninois pour les combattre. Elle est divisée en 3 axes :

Axe 1 : il décrit les principales caractéristiques cliniques de chacune des 9 MTN-C ainsi que les modalités de diagnostic et de prise en charge selon l'OMS. Il décrit également le statut épidémiologique mondial et Africain de chacune des MTN-C, selon les dernières données publiées par l'OMS.

Axe 2 : il détaille les principales stratégies d'intervention éditées par l'OMS pour la prise en charge des MTN-C.

Axe 3 : il présente l'organisation mise en place au Bénin pour lutter contre les MTN-C endémiques, ainsi que les freins rencontrés dans l'application de certaines des mesures.

Revue bibliographique de thèse :
**« Intervention strategies for the
management of tropical skin diseases:
the context in Bénin »**

Manuscrit en cours d'écriture

I. Introduction

According to the World Health Organization (WHO), neglected tropical diseases (NTDs) include 20 communicable diseases caused by bacteria, protozoa, helminths and fungi. These diseases are common in tropical and subtropical climates and affect more than one billion people, placing a huge annual financial burden on the economies of the countries in which these diseases are endemic [1].

These make the lives of populations at risk in neglected areas much more complicated, which has motivated the WHO to implement strategies for combating them. Integrated and streamlined approaches were launched after the first meeting of WHO global partners in 2007 [2]. Progress has since been made towards the treatment and elimination of many of these diseases [3]. In 2020, 600 million fewer people required NTD interventions than in 2010, and 42 countries had succeeded in eliminating at least one of these diseases [4]. These huge successes have been achieved since the publication of the first WHO roadmap for combating NTDs in 2012 [5].

However, the populations affected by these diseases are among the most vulnerable and marginalized in the world. For this reason, the burden of NTDs remains heavy despite the significant progress that has been made. The WHO therefore published a second roadmap in 2020. A final report will be published in 2031 to assess the overall impact of the actions planned in this roadmap [4].

Nine of the 20 diseases concerned have cutaneous manifestations in the form of plaques, swellings and ulcers on the face or body, and are therefore classified as “NTDs with skin manifestations”. These nine NTDs with skin manifestations are Buruli ulcer, leprosy, mycetoma, yaws, scabies, lymphatic filariasis, onchocerciasis, cutaneous leishmaniasis and post-kala-azar dermal leishmaniasis. Vital prognosis is poor in the absence of appropriate treatment, and these diseases can also lead to permanent disabilities associated with social stigma, promoting poverty, in survivors [2-5]. These diseases can, therefore, create psychological distress and lead to discrimination, often due to physical impairment and disfigurement [6].

All nine of these skin diseases can be cured by treatments well-defined by the WHO, but it is difficult to implement the tools required for their diagnosis in lower middle-income countries and to establish access to treatment and health care centers; as a result, the epidemiological status of these diseases is not regularly updated [7]. Little research is

performed and investment in the integration of care strategies for affected patients is limited [1, 8].

Benin is a country in West Africa in which several of these NTDs with skin manifestations, including Buruli ulcer in particular, are endemic [9]. The programs and centers for fighting these diseases in this country have made major efforts over the years to apply the recommendations of the 2012 WHO roadmap. Some plans have worked well, but others have run into difficulties.

In this review, we first describe the nine NTDs with skin manifestations. Then, we present the main intervention strategies recommended by the WHO in its latest report for the management of NTDs with skin manifestations. In the third part, we focus on how best to implement these strategies in Benin to minimize the burden of these diseases in the future. We then discuss the strategies that have been applied and those that have run into difficulties during the implementation in the country.

II. NTDs with skin manifestations

NTDs with skin manifestations mostly occur in humid tropical and subtropical regions, and frequently in very remote areas inhabited by low-income populations, in which access to clean water is difficult. All these conditions provide a favorable environment for the pathogens and their transmission. In this first part of the review, we will briefly present the nine NTDs with skin manifestations. **Figure 1** shows one manifestation type observed for each of these nine diseases. A summary of the main characteristics of each NTD with skin manifestations is presented in **Table 1**. The worldwide distribution of the number of new reported cases or prevalence of the NTDs with skin manifestations is presented in **Figure 2**, with a focus on Africa presented in **Figure 3**. The numbers of recently reported new cases for which data are available are shown, by WHO region, in **Table 2**.



Figure 1: Presentation of the nine NTDs with skin manifestations. Source WHO [7].

Buruli ulcer – Stage of ulceration (detachment of the edges). **Leprosy** – Borderline tuberculoid leprosy (Multiple pale plaques with loss of sensation). **Mycetoma** – Lumps (discharge of pus and blood from the openings of the fistulous tracts). **Scabies** – Small itchy nodules and scabious furrows. **Lymphatic filariasis** – Alteration with grainy appearance of the skin of a leg whose swelling indicates a chronic obstruction of the lymphatic vessels. **Cutaneous leishmaniasis** – Multiple lumps with scabs. **Post-kala azar dermal leishmaniasis** – Multiple smooth nodules. **Yaws** – A large nodule with a raspberry-like surface (papilloma). **Onchocerciasis** – Hypopigmented onchodermatitis (leopard skin).

Table 1. Summary of the main characteristics of each NTD with skin manifestations

| Disease | Main causal pathogen(s) | Region | Main clinical signs | Location of lesions | Age | Pain/Fever/Pruritus |
|--|--|--|--|--|--------------------|--|
| Buruli ulcer | <i>Mycobacterium ulcerans</i> | Africa (++), Asia, Americas, Western Pacific | Nodules, ulcers, swollen limbs, plaques | Mostly on limbs | Child/adult | No / no / no |
| Leprosy | <i>Mycobacterium leprae</i> | Africa, Asia, Americas, Eastern Mediterranean, Western Pacific | Nodules, ulcers, plaques | Anywhere - ulcers on the hands or feet | Child/adult | Loss of sensitivity / no, except in case of reactions / no |
| Mycetoma | <i>Eumycetoma: Madurella mycetomatis</i> <i>Actinomycetoma: Streptomyces somaliensis, Actinomadura madurae, Nocardia brasiliensis</i> | Africa, Asia, Americas, Eastern Mediterranean | Nodules, ulcers, swollen limbs | Limbs or trauma sites | Child/adult | No / no / no |
| Yaws | <i>Treponema pallidum ssp. pertenue</i> | Africa, Asia, Americas, Western Pacific | Nodules, ulcers, plaques | Anywhere – but often on limbs | Child/adult | No / no / no |
| Scabies | <i>Sarcoptes scabiei</i> | Africa (++), Asia (++), Americas (++), Europe, Eastern Mediterranean, Western Pacific (++) | Nodules | Anywhere – hands, external genitalia, feet, buttocks | Child/adult | No / no / yes |
| Lymphatic filariasis | <i>Wuchereria bancrofti</i> <i>Brugia timori</i> <i>Brugia malayi</i> | Africa (++), Asia (++), Americas, Eastern Mediterranean, Western Pacific (++) | Nodules, ulcers, swollen limbs (++), plaques | Swelling of the lower limbs and scrotum | Adults principally | Only in acute episodes / only in acute episodes / no |
| Onchocerciasis | <i>Onchocerca volvulus</i> | Africa, Americas, Eastern Mediterranean | Nodules, plaques | Anywhere - but mostly on limbs | Child/adult | No / no / possible |
| Cutaneous leishmaniasis | <i>Leishmania major</i> <i>Leishmania tropica</i> <i>Leishmania aethiopica</i> | Africa, Asia, Americas (++), Europe, Eastern Mediterranean (++) | Nodules, ulcers, plaques | Exposed areas | Child/adult | No / no / no |
| Post-kala-azar dermal leishmaniasis | <i>Leishmania donovani</i> | Africa, Asia, Eastern Mediterranean | Nodules, plaques | Anywhere | Adults principally | No / no / no |

.....To be continued

| Disease | Transmission | Type of diagnostic sample | Laboratory diagnostic tests | Medical treatment | Care and support measures |
|--------------------------------------|--|---|--|---|--|
| Buruli ulcer | Unknown, favored by contact with natural water sources | Swab, fine-needle aspiration, skin biopsy | PCR | Oral rifampicin + oral clarithromycin for 8 weeks | Wound dressing, physiotherapy as needed, depending on location and size of lesions |
| Leprosy | Close and prolonged human-to-human contact | Skin smear taken by incision or biopsy | Direct microscopic or histological examination | Treatment with a combination of rifampicin, dapsone and clofazimine for 6 months for paucibacillary leprosy or 12 months for multibacillary leprosy | Care of areas of insensitivity with regular wound inspection, physiotherapy as needed based on location and size of lesions, appropriate footwear, contact tracing |
| Mycetoma | Trauma, for example thorn prick | Biopsy | Microscopic or histological examination and grain culture | Depending on the species concerned: ong-term antibiotic therapy or antifungal treatment + surgery | Walking aid |
| Yaws | Skin-to-skin contact | Swab | DPP test and PCR | Single dose of oral azithromycin (second-line: benzathine penicillin injection) | Wound dressing, treatment of family and contacts |
| Scabies | Other household members who also have injuries | Clinical examination, dermoscopy, videomicroscopy | Search for the presence of mites, scratching | Permethrin cream, sulfur 4%, benzyl benzoate ointment (15%), oral ivermectin | Treatment of family and contacts |
| Lymphatic filariasis | Mosquitoes | Blood sample | Rapid diagnostic tests (thick drop taken during hours of maximum microfilaria circulation) | Oral diethylcarbamazine for 12 days + doxycycline for 4 to 6 weeks | Cleansing of the limbs, antibacterial/antifungal creams if necessary, physical exercise. Oral antipyretics and antibiotics during acute episodes. |
| Onchocerciasis | <i>Simulium</i> bites | Bloodless skin biopsy | Microscopic examination, PCR | Oral ivermectin, doxycycline in areas where Loa Loa is present unless the risk of side effects is known to be low | Emollients |
| Cutaneous leishmaniasis | <i>Phlebotomus</i> bites | Fine-needle aspiration | Microscopic examination | Stibogluconate, paromomycin, miltefosine, amphotericin B | Absence of care reported |
| Post-kala-azar dermal leishm. | <i>Phlebotomus</i> bites | Swab/biopsy | Microscopic examination, PCR | Miltefosine, amphotericine B | Absence of care reported |

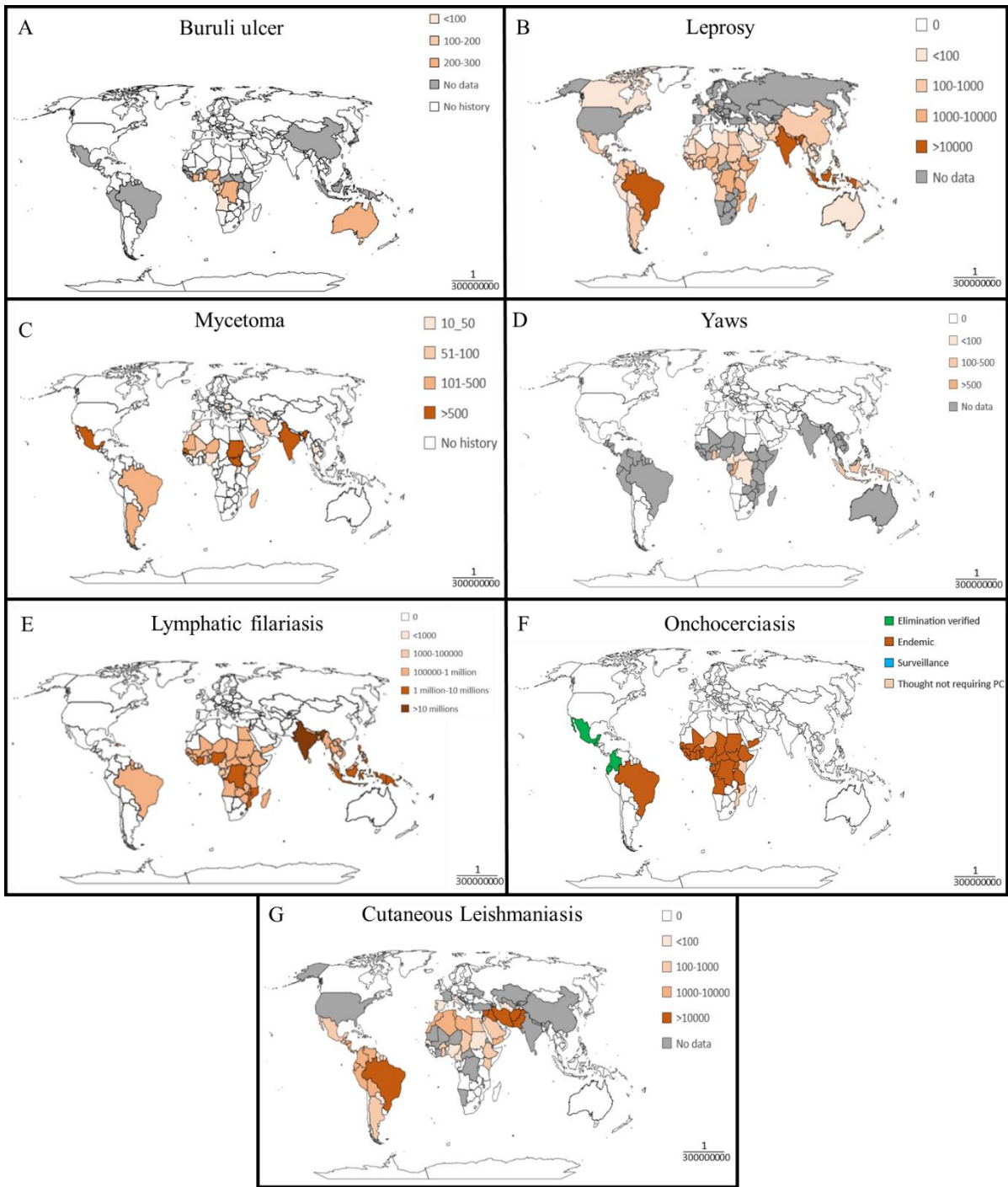


Figure 2. Worldwide distribution of the nine neglected tropical diseases with skin manifestations. **A.** new reported cases of Buruli ulcer (2021) [10], **B.** new reported cases of leprosy (2021) [11], **C.** new reported cases of mycetoma (2019) [12], **D.** new reported cases of yaws (2021) [13], **E.** prevalence of lymphatic filariasis (2019) [14], **F.** status of endemicity of onchocerciasis (2021) [15], **G.** new reported cases of cutaneous leishmaniasis (2021) [16].

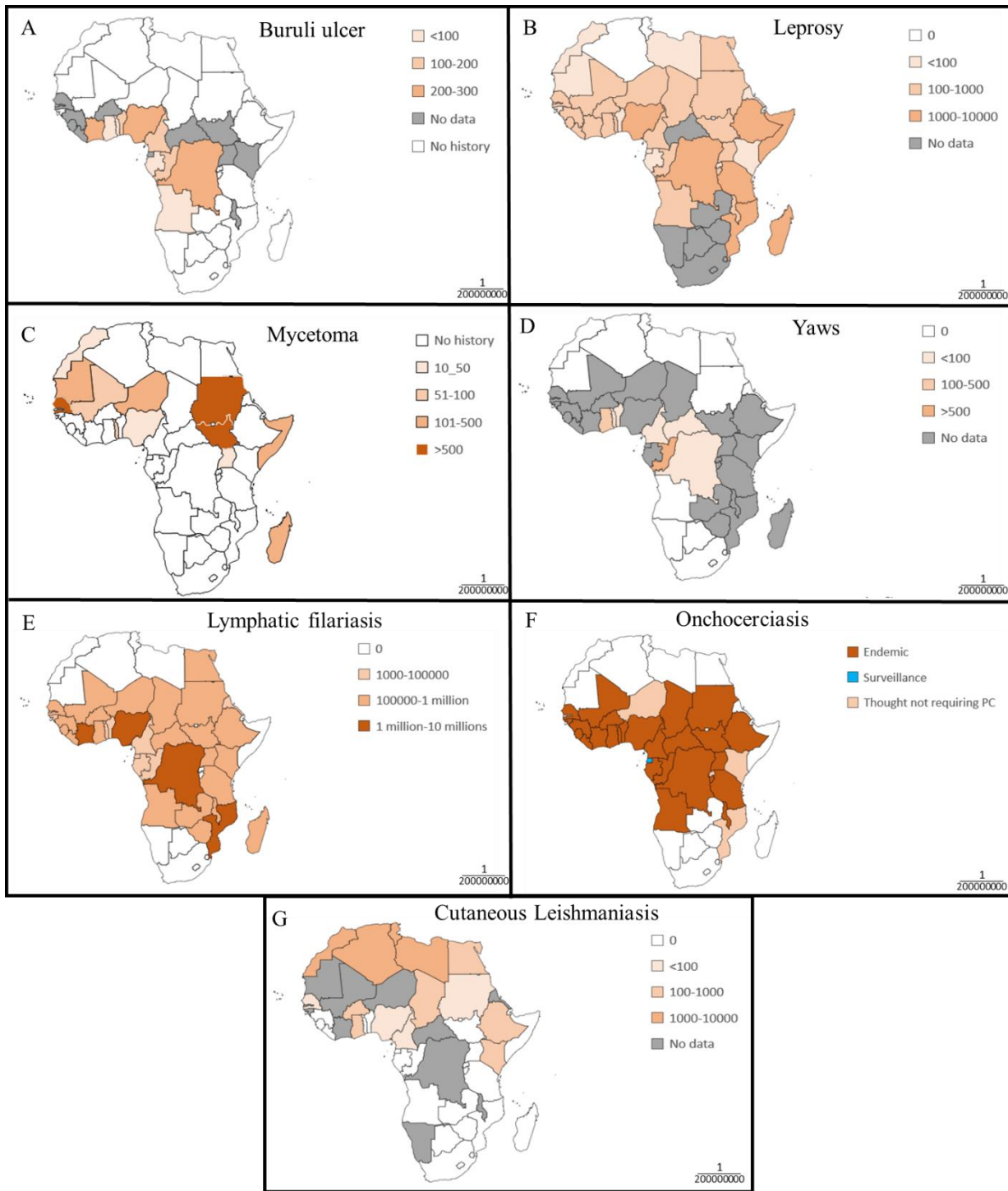


Figure 3. Distribution in Africa of the nine neglected tropical diseases with skin manifestations. **A.** new reported cases of Buruli ulcer (2021) [10], **B.** new reported cases of leprosy (2021) [11], **C.** new reported cases of mycetoma (2019) [12], **D.** new reported cases of yaws (2021) [13], **E.** prevalence of lymphatic filariasis (2019) [14], **F.** status of endemicity of onchocerciasis (2021) [15], **G.** new reported cases of cutaneous leishmaniasis (2021) [16].

Table 2. Number of new cases of Buruli ulcer, leprosy, cutaneous leishmaniasis, yaws and prevalence of lymphatic filariasis reported, by world region

| Skin NTDs (year of data collection) | WHO Region | Number of new cases/prevalence reported | Total | Source |
|--|-----------------------|--|----------------------------|---------------------------|
| Buruli ulcer (2021) | Africa | 1,370 | 1,661 | WHO [10] |
| | Americas | No data | | |
| | Eastern Mediterranean | No cases reported | | |
| | Europe | No cases reported | | |
| | South-East Asia | No data | | |
| | Western Pacific | 291 | | |
| Leprosy (2021) | Africa | 21,201 | 140,546 | WHO [11] |
| | Americas | 19,826 | | |
| | Eastern Mediterranean | 3,588 | | |
| | Europe | 14 | | |
| | South-East Asia | 93,485 | | |
| | Western Pacific | 2,432 | | |
| Cutaneous leishmaniasis (2021) | Africa | 9,165 | 221,731 | WHO [16] |
| | Americas | 37,502 | | |
| | Eastern Mediterranean | 174,861 | | |
| | Europe | 201 | | |
| | South-East Asia | 2 | | |
| | Western Pacific | No data | | |
| Yaws (2021) | Africa | 918 | 1,102 | WHO [13] |
| | Americas | No data | | |
| | Eastern Mediterranean | No data | | |
| | Europe | No cases reported | | |
| | South-East Asia | 169 | | |
| | Western Pacific | 15 | | |
| Lymphatic filariasis (2019) | Africa | 18,153,099 (prevalence) | 71,740,509 (prevalence) | Our World in Data [14] |
| | Americas | 574,105 | | |
| | Eastern Mediterranean | 1,449,575 | | |
| | Europe | 0 | | |
| | South-East Asia | 45,948,077 | | |
| | Western Pacific | 5,615,653 | | |

2.1. Buruli ulcer

Buruli ulcer, caused by *Mycobacterium ulcerans*, is the third most common mycobacterial disease worldwide after tuberculosis and leprosy [17]. This infection is characterized by painless skin lesions often in the form of raised nodules, plaques or edema, progressing to ulcers if left untreated. Buruli ulcers generally occur singly; they are large, with raised edges, a yellowish surface and a moist reddish-looking base [7]. Most affected individuals live in remote rural areas far from health centers, which delays the diagnosis and treatment of this disease. These delays in the initiation of treatment can result in long-term disabilities (25% of those affected), due to the need to amputate a limb or the occurrence of severe contractures after healing. These complications can lead to stigmatization of the patient, further increasing the burden of the disease [18].

Most cases are reported in Africa, and children under 15 years of age are the most affected. About 50% of infections occur on arms or legs [9]. The incubation period varies strongly and ranges from a few weeks to five months or more [19].

M. ulcerans is a bacterium associated with the aquatic environment [20]. No human-to-human transmission of Buruli ulcer has ever been reported and this disease is not, therefore, considered to be contagious [21]. The most plausible mode of transmission for *M. ulcerans* infection involves anthropogenic or natural skin-puncturing microtrauma. Decreasing exposure to insect bites, improving access to clean water for bathing and the prompt treatment of wounds are, therefore, concrete measures likely to interrupt transmission [22,23].

According to WHO guidelines, clinical cases of Buruli ulcer should be confirmed biologically by qPCR targeting a specific *M. ulcerans* sequence [24,25]. Medical treatment was long based on a combination of daily oral rifampicin and intramuscular streptomycin for eight weeks [17]. However, a recent phase III clinical trial concluded a fully oral combination of rifampicin and clarithromycin should be preferred for the early treatment of limited Buruli ulcer lesions, and this is now the recommended treatment [17,26]. Clinical strains of *M. ulcerans* resistant to rifampicin, streptomycin or clarithromycin have not yet been reported. Surgery and tissue grafts are often necessary for the treatment of large lesions [17].

2.2. Leprosy

Leprosy, also known as Hansen disease, is a chronic granulomatous infection mostly affecting the skin and peripheral nerves. It is generally caused by the "*Mycobacterium leprae* complex" which includes two intracellular bacteria: *M. leprae* and *M. lepromatosis* [27]. In 2000, the WHO considered leprosy to have been completely eliminated due to a decrease in prevalence to less than 1 case per 10,000 people [28], but this disease has not yet been completely eradicated. In total, 127 countries from six major world regions provided data for leprosy cases in 2020: 34 were in the WHO African Region, 22 in the Eastern Mediterranean Region, 19 in the Americas Region, 28 in the European Region, 13 in the Western Pacific Region and 11 in the South-East Asia Region [29].

The mean incubation period of this disease is five years, but symptoms may take 20 years or more to appear. The transmission pathway for leprosy is not fully understood, but there is evidence to suggest that individuals living in close contact with leprosy patients are at greater risk. Infection probably occurs through infectious aerosols created by coughing and sneezing, but also through direct contact. The reservoir for this disease was initially thought to be strictly human, but recent studies have suggested that anthroponotic and zoonotic transmissions may occur [30].

M. leprae displays an affinity for peripheral nerve cells and causes a loss of axonal conductance through nerve demyelination after Schwann cell attack, presented clinically as numbness [31]. A large proportion of individuals exposed to the bacterium do not go on to develop the disease. Indeed, disease development is dependent on many factors, including genetic predisposition and immune function. Leprosy diagnosis is essentially clinical, based on the presence of clinical signs, with no confirmation tests currently recommended. However, other tests, such as serological tests and immuno-enzymatic assays can be performed, with some polymerization chain reaction (PCR)-tests both effective and providing greater diagnostic precision, as reported for the PCR test targeting the RLEP sequence [32].

Treatment generally involves multidrug therapy to ensure that microbial resistance does not develop. The drugs used include dapsone, rifampicin and clofazimine. In combination, these drugs are the most effective and, if used early, can prevent extensive deformity and neurological disability [32]. Forms of resistance to anti-leprosy drugs have been reported in this bacterium since 1967, and multidrug resistance has also been observed on several continents, including Africa, Asia and South America; the GenoType LepraeDR kit is a recently developed tool that can be used to detect resistance to anti-leprosy drugs [33].

2.3. Mycetoma

Mycetoma is a chronic infection of the skin. It was previously known as “anthill foot” in the Southern province of Madura, and is, thus, often referred to as “Madura foot” [34]. Mycetoma is caused by either filamentous fungi (eumycotic) or bacteria (actinomycotic) present in the soil and plants. Actinomycetomas are caused by aerobic actinomycete bacteria belonging to the genera *Streptomyces*, *Actinomadura* and *Nocardia*; the species most commonly implicated are *Streptomyces somaliensis*, *Actinomadura madurae* and *Nocardia brasiliensis*. Eumycetomas are caused by fungi, with *Madurella mycetomatis* the species most frequently implicated [35].

Endemicity is highest in Sudan in Africa, Mexico and Venezuela in South America and India in Southern Asia. Cases have also been reported in other African countries and in Latin America [35]. The species causing mycetoma differ between countries, with *M. mycetomatis* the species most frequently implicated worldwide. Eumycetomas are more prevalent in areas of high rainfall, whereas actinomycetomas are more common in drier areas [34]. The incidence is higher in men than women, possibly due to a greater involvement of men in agricultural activities, in which the microorganism may enter the body through an injury when the person is working barefoot. The disease is more common in young adults and rare in children [34].

Regardless of the causal organism, the clinical features are almost uniform, presenting as a triad of firm, painless subcutaneous swelling, multiple sinus formation, and a purulent or seropurulent discharge containing grains [34].

The diagnosis of mycetoma is classically based on this triad. The causal agent can be identified by direct clinical examination, microscopy and culture of a grain on Petri dishes, or by molecular tests based on 16s RNA gene sequencing for actinomycetes and panfungal PCR for eumycetes. [36]. Choice of the most appropriate treatment is dependent on confirmation of the diagnosis and identification of the causal agent, as the treatment options for actinomycetoma and eumycetoma are different. Actinomycetomas are usually treated with a combination of antibiotics to prevent the development of drug resistance [36]. Eumycetomas are treated with antifungal agents for long periods -up to two or three years, but most respond poorly to drug treatment alone and surgery is generally also required [37]. It should also be noted that antifungal agents are expensive and difficult to obtain in some countries.

2.4. Yaws

Yaws is caused by the Gram-negative bacterium *Treponema pallidum ssp. pertenue* from the spirochete family. Humans are the only known reservoir for this bacterium [38]. The disease is mostly seen in children between the ages of two and 15 years and it spreads through direct skin-to-skin contact. Poor hygiene is a risk factor for transmission. Unlike syphilis, yaws is non-venereal and there is no mother-to-child transmission.

Clinically, the disease has two distinct stages, an early stage with cutaneous signs, followed by a period of latency and then a late stage in which yaws can manifest as gummy nodules and destructive bone damage [39]. *Treponema pertenue* is widespread in Africa, Asia and the South Pacific. The WHO has identified 12 countries in which this disease is endemic, eight of which are African: Benin, Central African Republic, Cameroon, Côte d'Ivoire, Republic of Congo, Ghana, Democratic Republic of Congo, Togo [39].

Diagnostic methods are limited because *Treponema pertenue* is not viable *in vitro*. Laboratory serological tests such, as rapid plasma reaction (RPR) and *Treponema pallidum* particle agglutination (TPPA) tests are widely used to diagnose treponemal infections (e.g., syphilis and yaws), but cannot distinguish between them [39]. Combination of these tests with PCR targeting the *tp0858* gene can be used for the definitive confirmation of cases of yaws [40].

Treatment is based on the administration of a single dose of azithromycin [39]. In 2012, the WHO implemented a policy of single-dose oral azithromycin and surveillance for the detection of cases in endemic areas, with the aim of eradicating yaws by 2020 [41].

2.5. Scabies

Scabies results from infestations of the mite *Sarcoptes scabiei*, an infectious organism that spreads through skin-to-skin contact or on clothing. The female mite burrows into the stratum corneum of the skin, where it lays its eggs, which then develop into larvae, pupae and adults, causing a relentless severe itch, especially at night [42]. Each year, 300 million people worldwide develop scabies.

This disease is widespread in Africa, South America, Australia and Southeast Asia, with children most frequently affected [42]. Observation of the rash is the method classically used for scabies diagnosis. The diagnosis is then confirmed by observing the mites, their eggs, and their feces by light microscopy or dermoscopy [43].

Topical applications of 5% permethrin cream once weekly for two weeks are generally effective [43]. Oral ivermectin treatment is also highly effective and has been approved in several countries [43], although topical permethrin applications are also used to deal with scabies outbreaks. [42,44]. However, these molecules are not always available and affected families are likely to spend a significant proportion of their monthly income on treatment with these drugs as a direct cost of scabies [45].

2.6. Lymphatic filariasis

This chronic parasitic infection is caused by three nematodes: *Wuchereria bancrofti*, *Brugia timori* and *Brugia malay* [46]. Mosquitoes from the genera *Aedes*, *Anopheles*, *Culex*, *Mansonia*, and *Ochlerotatus* serve as a vector for transmission to humans [47]. The mosquito deposits the larvae in the bloodstream, where they develop into adult worms after migrating to the lymph nodes. Larval growth and development disrupts lymphatic drainage by obstructing the lymph vessels. This inflammation leads to fibrosis, eventually resulting in elephantiasis.

This disease is the second leading cause of permanent deformity worldwide after leprosy, with 120 million people affected in 72 countries around the world. Half of these individuals are in their 30s or 40s, and there is a heavy male preponderance (male:female ratio of 10:1) [46].

This disease can be diagnosed through the microscopic identification of microfilariae in blood or skin samples during the hours of maximal microfilaria circulation [48]. The triple therapy currently recommended by the WHO consists of a single dose of a combination of ivermectin, albendazole and diethylcarbamazine. Topical flavonoids and coumarin have been found to be effective for reducing lymphedema. Treatment with antibiotics and antifungal creams is required, together with good skin hygiene, to prevent flare-ups of lymphangitis [47]. The Global Lymphatic Filariasis Elimination Program is ensuring massive drug delivery to populations in endemic areas with the aim of eradicating this disease [49].

2.7. Onchocerciasis

This disease is caused by *Onchocerca volvulus*, a filarial nematode transmitted by bites of the female black fly of the genus *Simulium damnosum*, which breeds in fast-flowing rivers, hence the common name of this disease: “river blindness”. In addition to blindness,

onchocerciasis also causes chronic dermatitis [50]. The definitive host of these filial nematodes is humans, whereas the larval stages develop in the black fly *Simulium*.

More than 99% of human infections occur in 31 African countries [15]. According to the WHO, at least 25 million people are infected worldwide, with vision loss affecting approximately 1.15 million people [51]. The prevalence and transmission of the disease are high in agricultural areas, especially near rivers, which serve as breeding grounds for the vector.

Clinical diagnosis is based on analyses of skin sections, which are incubated for 24 hours in a saline solution to encourage the emergence of cutaneous microfilariae, which are then observed and examined directly under a microscope. Serological test based on ELISA and western blotting for use with skin, tears and urine have also been developed for the detection of antibodies directed against the antigens of *Onchocerca volvulus*. PCR and ultrasound scans of subcutaneous nodules are also performed [50,52].

Treatment is based on a combination of oral ivermectin, which paralyzes or kills microfilariae, and doxycycline, which can kill the adult worm by killing *Wolbachia*, an endosymbiotic bacterium essential for adult worm survival and embryogenesis [50].

2.8. Cutaneous leishmaniasis

Cutaneous leishmaniasis is a skin infection caused by flagellated protozoa of the dermatropic type from the genus *Leishmania*: *Leishmania major*, *Leishmania tropica* and *Leishmania aethiopica*. The vectors of these protozoa are female dipterans from the genus *Phlebotomus* [16]. The WHO has estimated the number of new cases at 0.7 to 1.2 million per year [16].

This disease can affect all age groups, and usually occurs sporadically, especially during the displacement and settlement of populations [53]. The infection appears as rounded skin nodules, plaques or ulcers, the central part of which can give rise to a bleeding ulcer with a red base, often with raised edges [16].

Three major diagnostic methods of various degrees of accuracy have been used: parasitological examination (histopathology, microscopy and parasite culture), serology and molecular diagnostics [16, 54].

Local treatment is based on the use of sodium stibogluconate and meglumine antimonate, whereas general treatment is based on injections of pentavalent organic salts of antimony, pentamidine or amphotericin B deoxycholate [54].

2.9. Post-Kala azar dermal leishmaniasis

Post-kala azar dermal leishmaniasis (LDPK) is a cutaneous syndrome that appears as a late complication of visceral leishmaniasis [16, 55]. It results in the formation of macules and papules, initially around the mouth, which can then develop into nodules similar in appearance to those of leprosy. This form of leishmaniasis can occur at any age. About 5 to 20% of patients with visceral leishmaniasis develop LDPK and, in about 10 to 30% of patients, it appears within 6 to 12 months of recovery from visceral leishmaniasis.

Parasites isolated from patients with LDPK are biochemically and serologically identical to the *Leishmania donovani* (*L. donovani*) isolated in cases of visceral leishmaniasis. PCR can detect the parasite in more than 80% of cases. Treatment is based on sodium stibogluconate (20 mg/kg), administered for four months in India and for two months in Sudan. Liposomal amphotericin B and oral miltefosine have also been shown to be effective [55, 56].

III. Main intervention strategies for the management of NTDs with skin manifestations

3.1. Control, elimination, and eradication of NTDs with skin manifestations: the WHO roadmaps

In its first roadmap published in 2012, the WHO discussed strategies for managing all NTDs, including those with skin manifestations, through control, elimination or eradication [5]. The WHO defines these three terms as follows:

i) control of a disease is defined as a decrease in its incidence or prevalence, its morbidity and/or mortality to a locally acceptable level as a result of deliberate efforts, but with control measures still necessary because transmission continues. The NTDs with skin manifestations targeted for control are Buruli ulcer, mycetoma, cutaneous and scabies,

ii) elimination implies the stopping of disease transmission over a limited geographic area, although control measures may still be necessary to combat or prevent reintroduction of the disease from somewhere else. Elimination can also be that there are no manifestations of the disease. The NTDs with skin manifestations targeted for elimination are leprosy, lymphatic filariasis, onchocerciasis and visceral leishmaniasis, and

iii) eradication is defined as a permanent decrease to zero of the worldwide incidence of an infection caused by a specific pathogen, as a result of deliberate efforts, with no risk of reintroduction. The NTD with skin manifestation targeted for eradication is yaws [57-60].

In 2012, mycetoma and scabies had not yet been included in the list of NTDs. At the end of this first roadmap, in 2020, some targets had been achieved, but others had not, due to difficulties encountered in the field. The updating of the WHO roadmap with the addition of three more NTDs (including two NTDs with skin manifestations: mycetoma and scabies) led to the publication of a second roadmap in 2021, with the aim of pursuing the objectives already defined and achieving others by 2030 [57]. **Figure 4** shows a time scale of the history of the WHO strategy for NTDs with skin manifestations between 2012 and 2030 [4-5] and **Table 3** summarizes the main objectives for each NTD with skin manifestations set by the WHO in its first roadmap in 2012 [5], the status of each (objectives achieved or not) in 2020, and the new WHO goals set out in the second roadmap published in 2021 for achievement by 2030 [57].

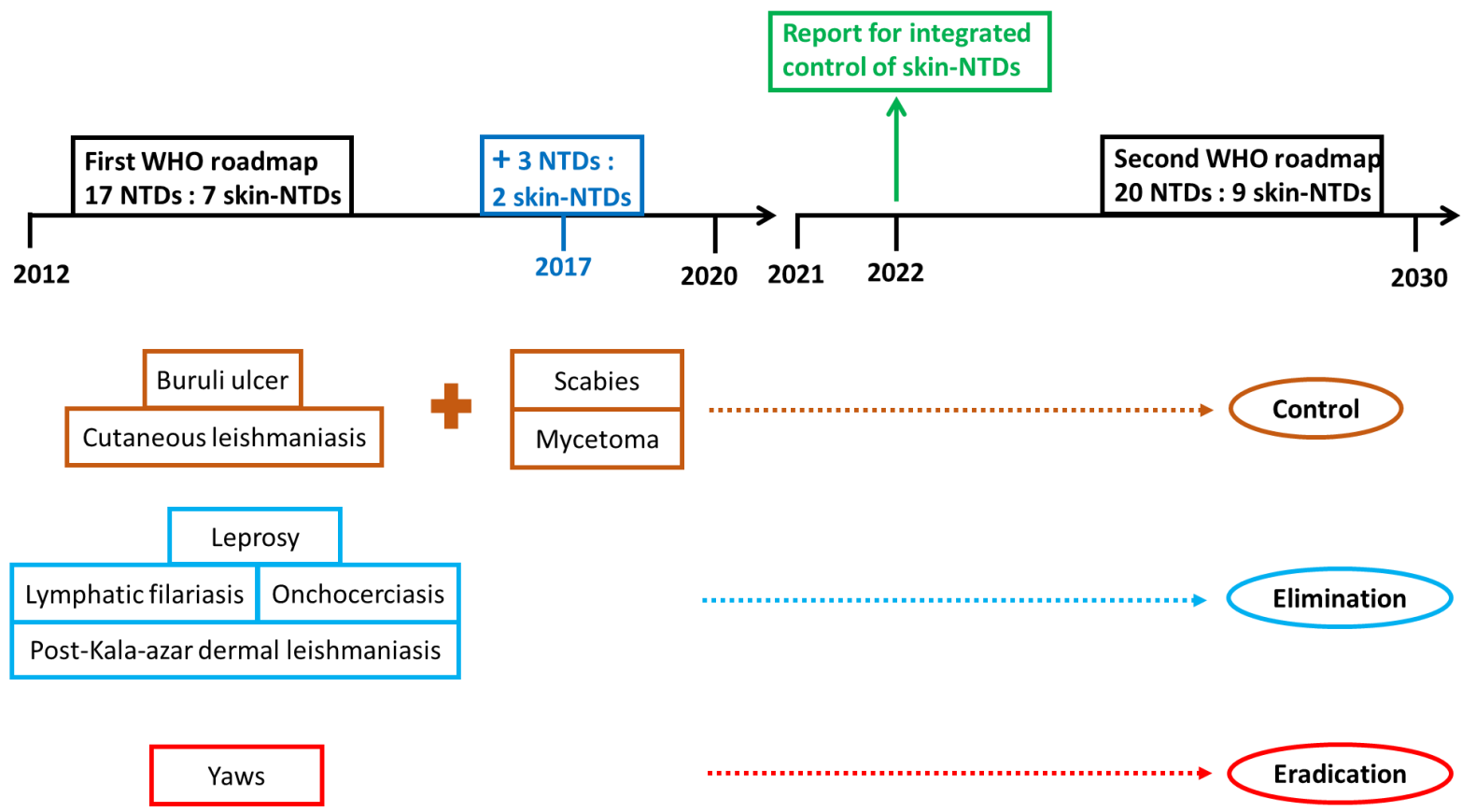


Figure 4. Evolution of the WHO strategic framework for the integrated control and management of neglected tropical diseases with skin manifestation [57].

Table 3. Summary of the objectives achieved in 2020, for each NTD with skin manifestations, according to the first WHO roadmap and the new objectives set in in the second roadmap for achievement in 2030.

| Disease | Objectives to be achieved according to the WHO roadmap 2012-2020 | Achievement of objective in 2020 | Objectives to be achieved according to the WHO roadmap 2021-2030 |
|-----------------------------|---|--|---|
| Buruli ulcer | <ul style="list-style-type: none"> ● Study completed and oral antibiotic therapy incorporated into control and treatment of Buruli ulcer ● 70% of all Buruli ulcer cases detected early and cured with antibiotics in all endemic countries in 2020 | <p>Achieved</p> <p>66% achieved</p> | <ul style="list-style-type: none"> ● <10% of cases in category III (late stage) at diagnosis ● >95% of laboratory-confirmed cases ● >98% of confirmed cases who have completed a full course of antibiotic treatment |
| Leprosy | <ul style="list-style-type: none"> ● < 1/million grade 2 disabilities in newly detected cases ● 0 grade 2 disabilities in new pediatric cases ● 0 countries with discriminatory laws | <p>1.5/million grade 2 disabilities in newly detected cases</p> <p>Estimation of 400-500 cases including all countries</p> <p>39 discriminatory laws in 17 countries</p> | <ul style="list-style-type: none"> ● 120 (62%) countries with 0 new autochthonous leprosy cases ● 62,500 new leprosy cases detected annually ● 0.12/million new cases with grade 2 disability ● 0.77/million new pediatric cases with leprosy |
| Mycetoma | Not applicable: mycetoma was not categorized as a WHO neglected tropical disease until 2017 | Not applicable | 15/30 (50%) countries in which mycetoma, chromoblastomycosis, sporotrichosis and/or paracoccidioidomycosis are included in national control programs and surveillance systems |
| Yaws | Eradication in 2020 (194/194 countries) | 1 certified; 106 with no previous history of yaws | 194 (100%) countries certified free of transmission |
| Scabies | Not applicable: scabies and other ectoparasitoses were not categorized as WHO neglected tropical diseases until 2017 | Not applicable | <ul style="list-style-type: none"> ● 194 (100%) countries having incorporated scabies management into the universal health coverage package of care ● 25 countries using MDA intervention in all endemic districts |
| Lymphatic filariasis | Global elimination (100%) as a public health problem in 2020 | 24% achieved | <ul style="list-style-type: none"> ● 58/72 (81%) countries validated for elimination as a public health problem |

| | | | |
|--|---|--|--|
| | | | <ul style="list-style-type: none"> ● 72 (100%) countries implementing post-MDA or post-validation surveillance ● 0 population requiring MDA |
| Onchocerciasis | <ul style="list-style-type: none"> ● 6 countries of the Americas eliminating transmission ● Elimination of transmission in Yemen by 2015 ● Elimination of transmission in Africa, where possible | <p>4 countries reached the goal</p> <p>Not achieved</p> <p>Not achieved</p> | <ul style="list-style-type: none"> ● 12 (31%) countries verified for interruption of transmission ● 34 countries that stopped MDA for ≥ 1 focus ● >16 countries that stopped MDA for $\geq 50\%$ of population ● >12 countries that stopped MDA for 100% of population |
| Cutaneous leishmaniasis | <ul style="list-style-type: none"> ● 70% of cases detected in the Eastern Mediterranean Region ● $\geq 90\%$ of all detected cases treated/managed according to guidelines | <p>Estimated ~20%</p> <p>Unknown</p> | <ul style="list-style-type: none"> ● 87/87 (100%) of countries in which 85% of all cases are detected and reported ● 95% of reported cases are treated |
| Post-kala-azar dermal leishmaniasis | <ul style="list-style-type: none"> ● 100% of districts/subdistricts on Indian subcontinent (Nepal, Bangladesh and India) with <1 case/10,000 population ● Countries validated as having eliminated visceral leishmaniasis as a public health problem (Bangladesh, India and Nepal) | <p>Bangladesh 100%, India 92%, Nepal 100%</p> <p>0: validation is required</p> | <ul style="list-style-type: none"> ● 64/75 (85%) of countries validated for elimination as a public health problem (defined as <1% case-fatality rate due to primary visceral leishmaniasis). ● 3/3 (100%) of countries in South-East Asia Region validated for elimination as a public health problem defined as <1 case (new and relapses) per 10,000 population at district level in Nepal and at subdistrict level in Bangladesh and India ● 100% in South-East Asia Region, post-kala-azar dermal leishmaniasis cases detected and treated |

MDA: mass drug administration

According to the WHO, core strategic interventions are required to control, eliminate or eradicate each of the NTDs with skin manifestations. These strategies have five elements: 1) preventive drug treatments, 2) water, sanitation and hygiene (WASH), 3) vector control, 4) veterinary public health and 5) case management. These applications of these core strategic interventions to NTDs with skin manifestations is presented in **Table 4**. The main critical actions required to achieve the objectives set by the WHO in its second roadmap for each NTD with skin manifestations are also summarized in **Table 5**.

3.2. Focus on the management of NTDs with skin manifestations by integration strategies

Nine years after the publication of the first WHO roadmap, many of NTDs have been reduced, with some close to elimination and eradication [5,57]. However, despite substantial progress in reducing the overall burden, many of the targets set for 2020 were not achieved, leading to a new roadmap taking previous positive experiences and difficulties into account [57]. This new roadmap recommends fighting NTDs in an integrated manner rather than focusing on specific diseases.

Several proposals have been made to optimize the strategies for achieving the targets set [57]:

- Social mobilization, with communities alerted to the importance of early diagnosis and treatment for suspected skin lesions
- Improvements in skin hygiene through regular washing to prevent these diseases and to protect against secondary wound infections.
- Reduction of the number of complications through the development of wound cleaning methods, massages, dressings and exercises to protect muscles or to maintain range of motion, practiced not only by health personnel, but also by the affected individuals themselves, and, in some cases, members of their families.
- Active case detection through activities such as mobile team consultations, to screen populations at risk, thereby increasing the number of cases, and to shed light on the underestimated prevalence of each disease, especially in very remote areas.
- Encouraging village volunteers to participate in the management and diagnosis and treatment activities for control.

- Implementation of appropriate training for health personnel, such as doctors, nurses and community health workers, to ensure a good knowledge of NTDs with skin manifestations.
- Implementation of the necessary training materials and support for the local laboratories involved in diagnosing these diseases. This should be the responsibility of the health ministries of endemic countries, which should also develop a national policy that, with technical support from the WHO and other external actors, can help fight these diseases.
- Patient care at specialist centers for complicated cases requiring surgical interventions. These centers must be supported at all levels by the public health system.
- Apply strategies that consist of mass drug administration for diseases possible to eradicate.
- Ensure access to better nutrition for all these endemic populations to boost their immunity.
- Strategies for reducing stigma and improving the mental health and quality of life of those affected.
- Approaches involving collaboration between human and animal health systems to prevent or mitigate certain NTDs with skin manifestations that have transmission vectors. These strategies will target the animal reservoir and its ecosystem.
- Promotion of a mobile health approach using smartphone applications and other wireless technologies, developed by the WHO to facilitate the diagnosis of NTDs with skin manifestations through the selection of signs and symptoms and the use of an automated algorithm in a very recent application [1]. However, this strategy requires an operational internet network, which has proved problematic in remote areas.
- A robust integrated routine surveillance system for all NTDs with skin manifestations in which data are sent to the national health information system via reporting forms or the internet, for continuous evaluation of the impact of these interventions on NTDs with skin manifestations.

Figure 5 summarizes and represents the connections between the different actors in endemic countries for integrating the main strategies.

We will now focus on Bénin, a country involved in the management of NTDs with skin manifestations in which efforts to integrate NTD control programs into national or regional priorities have paid off. We also discuss the obstacles faced by this country in the application of control programs.

Table 4 : The core strategic interventions recommended by WHO for each NTD with skin manifestations

| Disease | Preventive drug treatment | WASH | Vector control | Veterinary public health | Case management | Others |
|---------------------|---|--|----------------|--------------------------|--|---|
| Buruli ulcer | Not applicable | Not applicable | Not applicable | Not applicable | Recommended treatment: rifampicin and clarithromycin or rifampicin and moxifloxacin, Hygienic wound care | Early diagnosis essential for morbidity management (surgery, wound and lymphedema management, physiotherapy and long-term rehabilitation) |
| Leprosy | Post-exposure prophylaxis administered to all contacts of detected and consenting cases (single-dose rifampicin reduces the risk of leprosy among contacts by 60%) | Access to clean water for wound care and routine self-care including daily soaking of hands and feet to prevent secondary disabilities; ensure hygiene, water and sanitation in health care facilities | Not applicable | Not applicable | Early detection of cases, multidrug therapy lasting 6 to 12 months with dapsone, rifampicin and clofazimine, periodic monitoring, detection and treatment of immunological reactions (type 1 and 2) and nerve damage, management of adverse drug reactions, counseling and psychological first aid, prevention of disability, wound care and management of disability including self-care, rehabilitation to optimize functioning of the individual in the community | Interventions addressing stigmatization and discrimination. Counseling and health education are essential to help leprosy patients, their families and communities to complete treatment and cope with physical and mental consequences |
| Mycetoma | Not applicable | Personal hygiene and self-care of the affected limbs to avoid secondary infections | Not applicable | Not applicable | Long-term antibiotic combinations for eumycetoma, combined antifungal agents and surgery for actinomycetoma, wound care (cleaning, dressing, disinfecting) | Wearing protective clothes (long sleeves and trousers) and closed-toe shoes |
| Yaws | Single-dose oral azithromycin | Personal hygiene | Not applicable | Not applicable | Azithromycin used as first-line treatment, intramuscular benzathine benzylpenicillin used for second-line treatment and for those with | Not applicable |

| | | | | | | |
|-----------------------------|--|--|---|----------------|---|--|
| | | | | | proven resistance to azithromycin, patients should be examined after 4 weeks; more than 95% cases are completely healed | |
| Scabies | MDA using oral ivermectin and topical scabicides | Hygiene measures | Not applicable | Not applicable | Topical scabicides (permethrin, benzyl benzoate, malathion and sulfur ointment), oral ivermectin, Treatment of all household contacts, specialist case management of crusted scabies cases | Not applicable |
| Lymphatic filariasis | MDA stops the spread of infection through WHO-recommended regimens of albendazole, diethylcarbamazine and ivermectin | Hygiene of affected limbs is essential for morbidity management. Sanitation improvements can reduce vector breeding habitats | Vector control to reduce transmission; type of vector control varies according to parasite–vector species and local ecology (e.g. use insecticide-treated nets in areas where <i>Anopheles</i> is the primary vector) | Not applicable | Skin care and hygiene, exercises and elevation to prevent severity and progression of lymphedema, treatment for episodes of adenolymphangitis, surgery to cure hydrocele, antifilarial medicines for treatment of infection, helping communities to complete treatment and cope with physical and mental consequences | Not applicable |
| Onchocerciasis | Once to twice yearly community-based MDA with ivermectin with adequate coverage for 10 or more years; may further increase frequency in selected circumstances | Not applicable | Environmentally safe spraying of insecticides at black fly habitats and larval breeding sites, new strategy of removing trailing vegetation in rivers under development | Not applicable | Ivermectin treatment to manage symptoms, doxycycline for cure in appropriate circumstances, management of visual impairment | Where loiasis is co-endemic, systems to manage serious adverse events must be in place; new strategies against loiasis are being developed |

| | | | | | | |
|--|----------------|----------------|---|---|---|--|
| Cutaneous leishmaniasis | Not applicable | Not applicable | Insecticide spraying, insecticide-treated nets and environmental management | Rodent control | Local treatment includes pentavalent antimony drugs, paromomycin, cryotherapy and thermotherapy. Systemic medicines include liposomal amphotericin B, pentavalent antimony drugs and miltefosine. | Early diagnosis (rapid diagnostic tests combined with clinical signs) and prompt treatment |
| Post-kala-azar dermal leishmaniasis | Not applicable | Not applicable | Insecticide spraying, insecticide-treated nets and environmental management | Strategies such as vaccines evaluated in dogs | Pentavalent antimony drugs liposomal amphotericin B paromomycin and miltefosine are used for treatment | Early diagnosis (rapid diagnostic tests combined with clinical signs) and prompt treatment |

MDA: mass drug administration

WASH: water, sanitation, hygiene

Table 5: The main WHO critical actions for reaching 2030 targets according to the second roadmap.

| Disease | WHO critical actions to reach 2030 targets |
|---------------------|--|
| Buruli ulcer | <ul style="list-style-type: none"> ● Build capacity of health workers to clinically diagnose and treat the disease and community health workers to detect and refer cases for early treatment, furthering integration of NTDs with skin manifestations. ● Develop rapid diagnostic tools for use in public health and community centres to ensure early diagnosis, reduce morbidity and confirm cases. ● Create comprehensive surveillance systems in all endemic countries, including micromapping, to improve targeting and integrating interventions with those for other NTDs in coendemic areas to improve case detection |
| Leprosy | <ul style="list-style-type: none"> ● Update country guidelines to include use of single-dose rifampicin for postexposure prophylaxis for contacts; advance research on new preventive approaches. ● Continue investment into research for diagnostics for disease and infection; develop surveillance strategies, systems and guidelines for case-finding and treatment; ensure resources for validation. ● Ensure the supply of medicines, including access to multidrug therapy, prophylactic drugs, second-line treatments and medicines to treat reactions; monitor adverse events (pharmacovigilance) and resistance. ● Ensure capacity for case-finding (screening, diagnosis), treatment and surveillance; integrate with primary care, skin and other NTDs, TB and/or other programs where appropriate. ● Combat stigmatization and discrimination to ensure access to services and inclusion in society; ensure human rights of people with leprosy are respected. |
| Mycetoma | <ul style="list-style-type: none"> ● Develop differential rapid diagnostic tests and effective treatment, and establish surveillance for case detection and reporting ● Develop a standardized field manual for diagnosis and treatment, and ensure proper training of healthcare workers. ● Provide access to affordable diagnosis and treatment. |
| Yaws | <ul style="list-style-type: none"> ● Start mass drug administration (MDA) in all endemic areas after mapping ● Strengthen active and passive surveillance ● Ensure effective, efficient integration and/or co-implementation with other programs and sectors (e.g. integrated management of NTDs with skin manifestations) ● Increase funding and advocacy for yaws eradication, including securing longer-term commitments and increasing the priority of yaws as suitable for preventive drug treatment and as a NTD with skin manifestations. |

| | |
|--|--|
| Scabies | <ul style="list-style-type: none"> ● Develop guidance and tools for mapping in endemic countries to estimate the burden of disease. ● Develop guidance for the implementation of preventive chemotherapy. ● Create an advocacy and funding plan; secure financing for ivermectin and topical treatments; advocate for inclusion in universal health coverage. |
| Lymphatic filariasis | <ul style="list-style-type: none"> ● Start MDA in all endemic districts and strengthen it in all settings; implement improved interventions where appropriate (e.g. three-medicine treatment in settings that qualify; strategies for hotspots). ● Improve capacity for morbidity management and disability prevention; prioritize in primary health care and as part of universal health coverage. ● Improve diagnostics, strengthen criteria for stopping MDA, establish post-MDA and post-validation surveillance standards; update guidelines with new tools and strategies as appropriate. |
| Onchocerciasis | <ul style="list-style-type: none"> ● Start MDA in all endemic areas after mapping, improve delivery of current MDA programs, and implement alternative strategies where appropriate ● Develop improved diagnostics to facilitate mapping and decisions to eliminate transmission; develop improved diagnostic strategy for loiasis; increase program capacity to perform entomological and laboratory diagnostics. ● Develop a macrofilaricide and diagnostic or other elimination strategies to accelerate interruption of transmission; design a case management strategy; develop and implement elimination strategies for areas where loiasis is endemic but onchocerciasis is hypoendemic. |
| Cutaneous leishmaniasis | <ul style="list-style-type: none"> ● Develop and scale up easy-to-administer oral or topical treatment that could be used in health centers. ● Improve the affordability and sensitivity of rapid diagnostic tests for the detection of cases, and the availability of treatment ● Estimate the burden of the disease by improving surveillance, and establish a patient database to ensure effective monitoring of the impact of control interventions. |
| Post-kala-azar dermal leishmaniasis | <ul style="list-style-type: none"> ● Enable early detection to ensure prompt treatment, through, for example, active case detection. ● Ensure the supply of medicines to ensure prompt access to treatment, especially during outbreaks, and especially for children and young adults, who make up 50–70% of the affected population ● Develop more effective and user-friendly treatment and diagnostics, especially for East Africa. |

MDA: mass drug administration

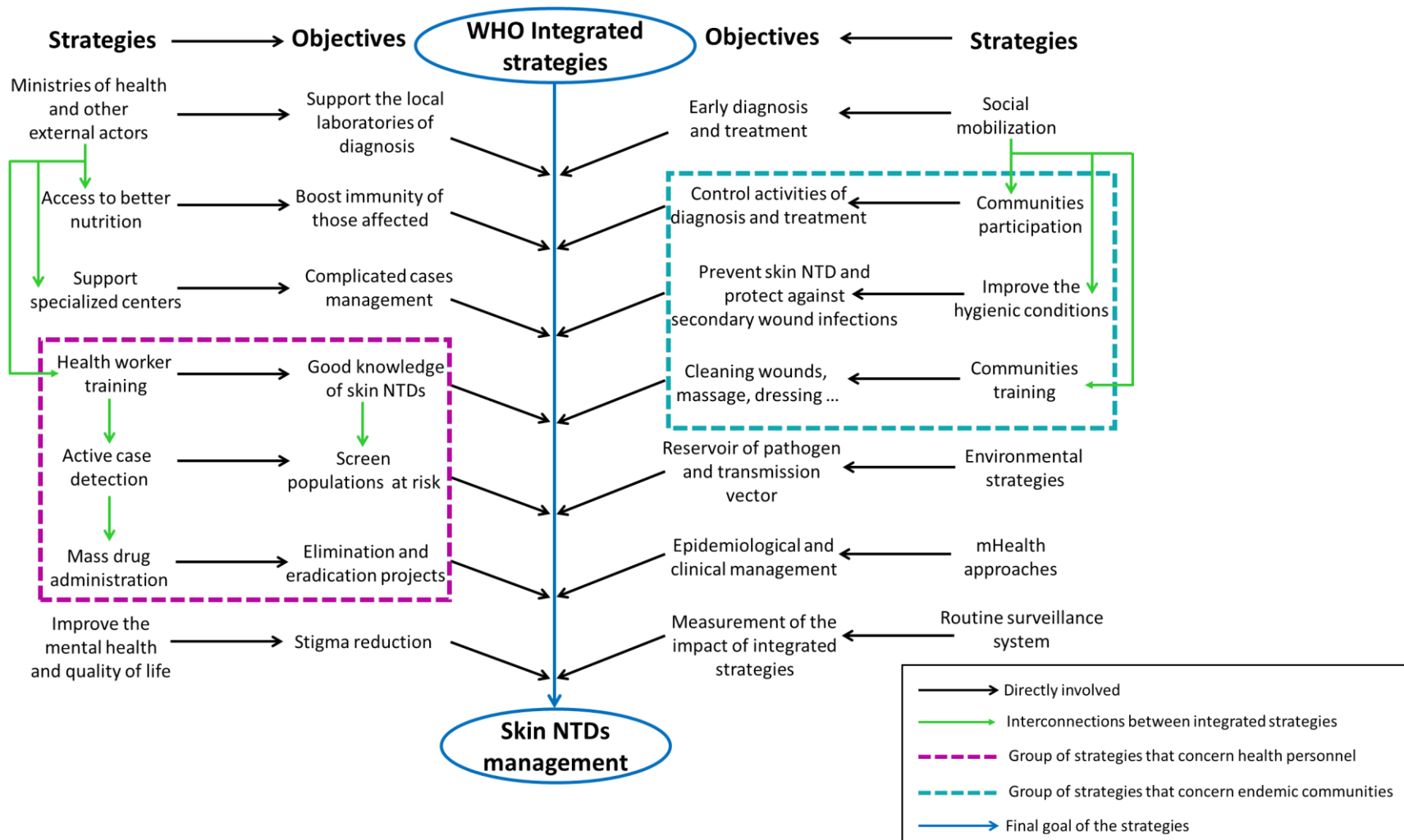


Figure 5. Presentation of the main integrated strategies that target the neglected tropical diseases with skin manifestation.

IV. Context of Benin regarding NTDs with skin manifestations

4.1. General information on Benin

Benin is a country located in West Africa. It covers an area of 114,763 square kilometers divided in 12 departments and subdivided into 77 communes, 546 districts and 5,295 villages [61]. The country has about 13 million inhabitants and almost 54% of the Beninese population live in rural areas, 45% reside in urban areas. Children under 14 years old represent among 42% of the population. Concerning the economic activities, they are mainly represented by agriculture and fishing. The country is located in the intertropical zone between the equator and the tropic of cancer thus he has a hot and humid climate which favors the spread of several infectious diseases, especially during the floods that threaten the low valleys and deltaic areas of the major rivers [61]. Among the 9 NTDs with skin manifestations, those which are considered endemic in Benin are Buruli ulcer, leprosy, lymphatic filariasis and onchocerciasis [61].

In Benin, ethnic diversity is a richness and does participate actively in the implementation of different strategies to fight against NTDs. Various social actors are involved in these strategies, such as opinion leaders, local elected officials, religious leaders who participate in social mobilization and help to remove reluctance, as well as teachers who help to raise awareness [62]. In addition, community relays are involved in screening, mass drug administration and home management of certain infectious complications [62, 63]. Social actors may travel great distances to reach the people to be treated, when the latter live in remote areas that are difficult to access [61].

4.2. Organization of strategies for the control of NTDs with skin manifestations

Benin has organised the management of skin NTDs at a country level, according to the pathology. Thus, Buruli ulcer and leprosy are managed by the national leprosy and Buruli ulcer control program (PNLLUB, - Programme National de Lutte contre la lèpre et l'Ulcère de Buruli), whereas the national communicable disease control program (PNLMT, Programme National de Lutte contre les Maladiers Transmissibles) supports the two other skin NDTs endemic in the country, lymphatic filariasis and onchocerciasis. Here is the organization of these structures:

4.2.1. Management of Buruli ulcer and leprosy

In 1997, Benin has developed a national program to fight Buruli ulcer (PNLUB - Programme National de Lutte contre l'Ulcère de Buruli). One of the priorities of this program was the establishment of a surveillance system against this disease [64]. For this, Benin has set up 4 Buruli ulcer screening and treatment centers (CDTUB – Centre de Dépistage et de Traitement de l'Ulcère de Buruli), in Allada (Atlantic department), Lalo (Couffo department), Zagnanado (Zou department) and Pobe (Ouémé department). These centers were built in the south of Benin, endemic for Buruli ulcer. Each center had as its primary mission the screening and management of Buruli ulcer according to the recommendations of the PNLUB [60].

From 2004, the system has evolved to integrate leprosy in its activities. This led to a remarkable progress, the program and the centers were then respectively called national leprosy and Buruli ulcer control program (PNLLUB, - Programme National de Lutte contre la lèpre et l'Ulcère de Buruli) and leprosy and Buruli ulcer screening and treatment center (CDTLUB – Centre de Dépistage et de Traitement de la Lèpre et de l'Ulcère de Buruli). Besides, 8 leprosy treatment centers (CTAL - Centre de Traitement Anti Lèpre) were constructed to cover all the country in order to optimize the management of patients. The functioning of all these structures has been possible thanks to the WHO, the Raoul Follereau Foundation and the ANESVAD Foundation, which support research on leprosy and Buruli ulcer in Benin [61,65,66].

Thanks to the efforts put by the control program and field structures in collaboration with the different partners, the number of Buruli ulcer new cases registered decreased gradually over the years from over 1,000 in 2007, to 240 cases in 2019 [10]. In 2021, 178 cases were detected, probably linked to COVID-19 (**Figure 6C**). The distribution of the number of new cases of Buruli ulcer reported in each department of Benin are presented in **Figure 6A**.

Concerning leprosy, the number of new cases recorded in Benin was around 400 in 2005 and 2006. Then, a gradual decrease was observed over the years with only 77 new cases detected in 2021 (**Figure 6D**) thus giving a case detection rate per 1,000,000 population of 5.92. This decrease can also be associated with the success of the large leprosy control program activities in this country for years [10]. Availability and good accessibility to treatment was also observed, with 100% of leprosy patients who were treated (data 2015) and 82% of them who were monthly supervised in the peripheral health facilities [10]. The

Distribution of the number of new cases reported of leprosy in each department of Benin in 2015 is presented in **Figure 6B** [61].

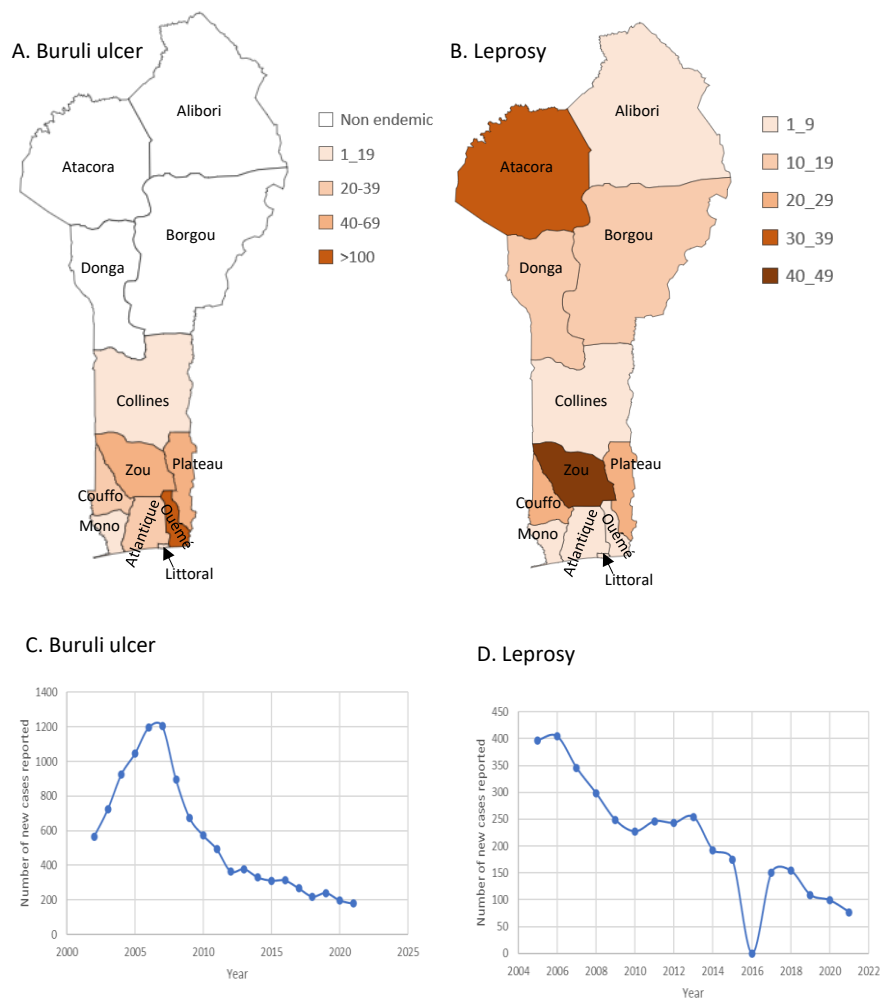


Figure 6. Distribution of the number of new reported cases of endemic neglected tropical skin diseases in each department of Benin and the evolution over the years. **A.** New reported cases of Buruli ulcer (2015) [61], **B.** New reported cases of leprosy (2015) [61], **C.** Evolution of the number of new reported cases of Buruli ulcer between 2002 and 2021 [10], **D.** Evolution of the number of new reported cases of leprosy between 2005 and 2021; no data available for 2016 [11].

4.2.2. Management of lymphatic filariasis and onchocerciasis

Beside the PNLLUB, Benin has set up a national communicable disease control program (PNLMT) responsible for taking care of several other endemic communicable diseases in the country, including the endemic NTDs with skin manifestation lymphatic filariasis and onchocerciasis. **Figure 7** represents the general health system of Benin with the programs and the structures that support NTDs with skin manifestations in the country.

Concerning lymphatic filariasis, in 2000, global prevalence was 2.03%, with a variation of 0 to 12.5% depending on the village [61]. Following this observation, a plan to eliminate lymphatic filariasis was initiated in 2002, by the PNLMT, with the guidance of WHO. From 2002 to 2011, 30 communes were enrolled in the mass treatment in a progressive manner. In 2012, 23 of the 30 communes on treatment stopped the mass treatment after the transmission assessment survey, which was conclusive (0 positive cases in 5 evaluation units and only 1 positive case in one evaluation unit where the threshold not to be exceeded is 20). As of 2013, all of the remaining 25 endemic communes have been placed on mass treatment except Cotonou and Porto-Novo, two communes that have shown not be endemic for lymphatic filariasis [61, 67].

Concerning onchocerciasis, control operations began in 1974 with the Onchocerciasis Control Program in West Africa (Programme de Lutte contre l'Onchocercose en Afrique de l'Ouest), supported by WHO. In 1988, the introduction of ivermectin as a treatment was launched in Benin, then vector control by spreading larvicides in watercourses was applied. Today, after more than 40 years of struggle, the prevalence of onchocerciasis is less than 5% in all the villages. The disease remains however endemic in 51 of the 77 communes (data 2015) [61].

The main lymphatic filariasis and onchocerciasis control activities triggered by the control programs in Benin and their endemic status before and after these activities are illustrated in **Table 6**. This table also presents the strategies to be maintained in the future in order to bring these 2 diseases under control.

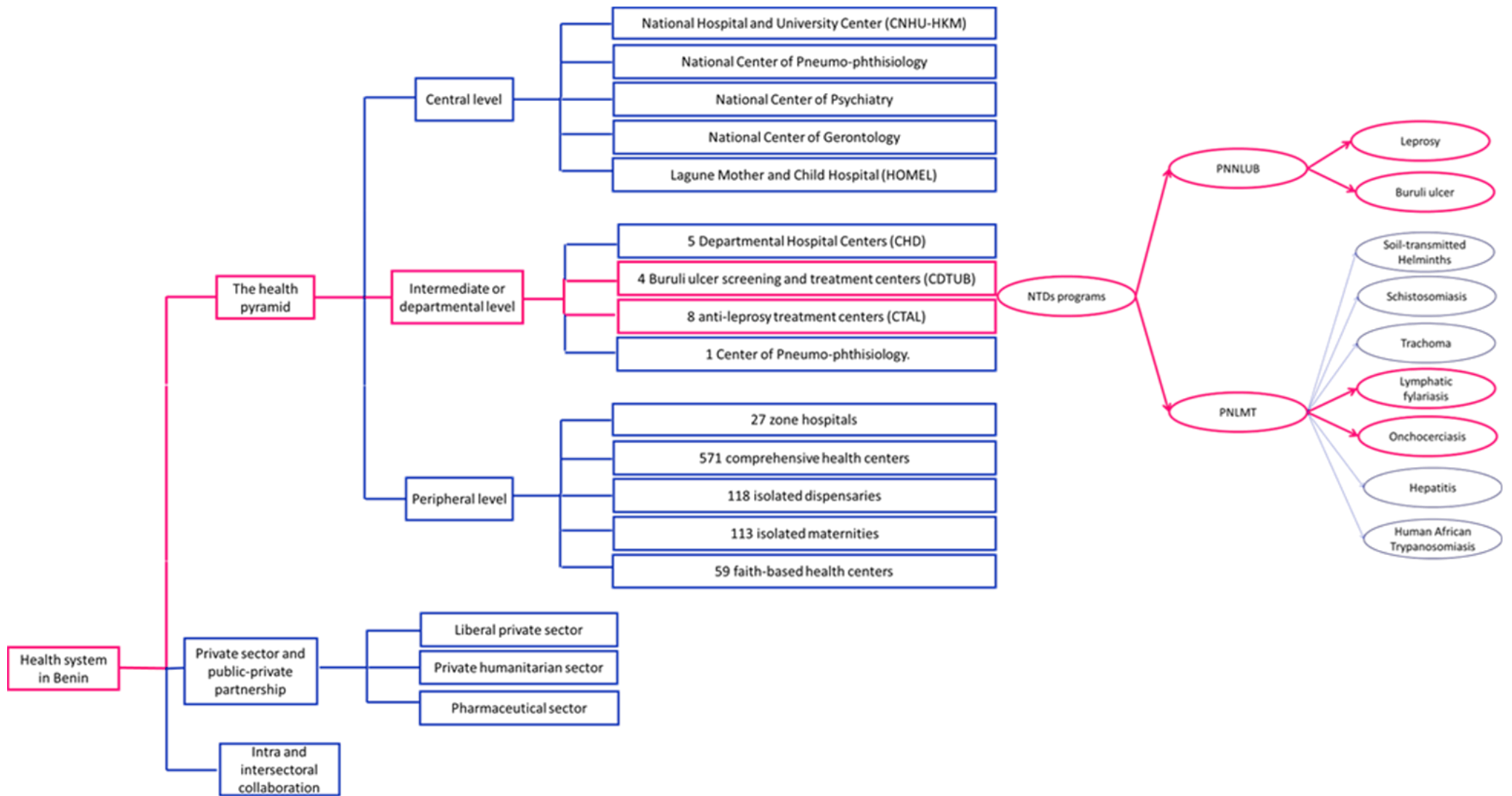


Figure 7. Tree representing the general health system of Benin with its different structures as well as the programs to fight neglected tropical diseases with skin manifestation in the country.

Table 6. Endemic status of lymphatic filariasis and onchocerciasis before and after control activities in Benin.

| | Endemic status in Benin before activities | Support program (year of start of activities) | Main activities | Endemic status in Benin after control activities | Perspective |
|-----------------------------|---|---|--|--|--|
| Onchocerciasis | <ul style="list-style-type: none"> - Endemic area: 81,000 km² - Endemic settlements: 51/77 - Prevalence: 25% to 98% - Annual transmission potential: > 800 infective larvae per person and per year | Onchocerciasis Control Program in West Africa (OCP) (1974) | <ul style="list-style-type: none"> - Vector control by spreading larvicides in water courses - Treatment with ivermectin | <ul style="list-style-type: none"> - 2000 to 2002: annual transmission potential < 100 and prevalence < 5% in most villages - In 2015: 51 endemic settlements under control with residual activities after: <ul style="list-style-type: none"> ● Treatment with ivermectin ● Epidemiological surveillance ● Entomological surveillance | <ul style="list-style-type: none"> - Treatment with ivermectin - Epidemiological surveillance - Entomological surveillance |
| Lymphatic filariasis | <ul style="list-style-type: none"> Cartography 2000: - Endemic settlements: 50/77 - Prevalence: 0% to 12.5% - High prevalence rates in Mono, Couffo , Ouémé, Plateau, Zou, Collines, Atacora and Donga | A lymphatic filariasis elimination plan has been implemented since 2002 | Mass drug administration | <ul style="list-style-type: none"> - 2002 to 2011: 30 settlements have been enrolled in mass drug administration -2016 : only 25 district under treatment | <ul style="list-style-type: none"> - Mass drug administration, Epidemiological surveillance - Set up a management system for the disabilities linked to this disease - Perform a treatment discontinuation study in the endemic municipalities that have gone through six rounds of treatment |

4.2.3. Management of other NTDs with skin manifestation in Benin

No case of cutaneous leishmaniasis and post-kala-azar dermal leishmaniasis has been reported in Benin [16]. Concerning yaws, the disease is currently not considered endemic in the country, even if in 2021, 198 suspected cases have been reported, but only 2 were confirmed [13]. Regarding mycetoma, between 0.01 and 0.1 cases/100,000 inhabitants were reported in Benin in 2021 [68]. Unfortunately, there is currently no program or structure for the control of mycetoma in Benin, while physicians are faced with the problem of treatment, which is very long and expensive, and the lack of an ideal diagnostic tool. As a consequence, this disease is often neglected. It is therefore urgent to integrate mycetoma in national programs in order to provide an adequate management of patients.

Scabies was only categorized a neglected tropical disease by WHO in 2017 [69], mainly because this disease should not lead to disabilities compared to Buruli ulcer and other NTDs with skin manifestations. Therefore, it is not yet well integrated among the strategies of control programs in Benin. However, according to recent studies, scabies is becoming the leading skin NTD in the country. This disease constitutes a real public health problem because of its high prevalence, and its high morbidity in children [61,70]. Thus, there is an urgent need to create an advocacy and funding plan but also to secure financing for ivermectin and topical treatments since currently, this treatment is reserved for onchocerciasis in the country.

V. Conclusion

This review provided an overview of all skin NTDs reported to date and their control strategies as recommended by the WHO. It also described the case of Benin, a West African country endemic for some of these skin NTDs and the national programs and structures for screening, treatment and prevention set up in this country for the management of infected patients.

This work has also enabled us to understand the difficulties encountered in the Beninese field in applying WHO strategies, as well as the solutions that may have been proposed by local teams. Similar work should be carried out by the other endemic countries of West Africa, in collaboration with health workers in the field and community relays, in order to help the WHO and other partners to find appropriate solutions, and thus, lead to a definite reduction in the burden of these diseases.

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**PARTIE 2 : Focus sur l'ulcère de Buruli
et les autres maladies tropicales
négligées endémiques au Bénin**

Avant-propos

L'ulcère de Buruli est une infection cutanée humaine due à *Mycobacterium ulcerans*, une mycobactérie environnementale. Cette pathologie sévit surtout dans les régions tropicales humides, notamment à proximité de régions marécageuses ou inondables. On la retrouve en particulier en Afrique de l'Ouest, où elle est endémique (19). En l'absence de prise en charge, l'infection conduit souvent à une destruction étendue de la peau et des tissus mous avec formation d'ulcérations importantes, généralement sur les membres, et à l'origine d'importantes incapacités fonctionnelles (20,21). La destruction tissulaire est liée à la production d'une toxine, la mycolactone. Cette toxine est le principal facteur de virulence de la bactérie, elle confère un caractère non douloureux à l'ulcère (22,23). Cette maladie touche majoritairement les enfants de moins de quinze ans et constitue une menace pour la santé publique dans de nombreuses régions en raison des conséquences socio-économiques qu'elle engendre (20). En 1998, l'Organisation Mondiale de la Santé a déclaré l'ulcère de Buruli «maladie émergente» (24) et en 2003, la maladie est devenue une «maladie tropicale négligée». Le traitement associe une bithérapie antibiotique si la prise en charge de l'ulcère est précoce, à la condition d'avoir un accès au dépistage et au soin. En cas de formes plus avancées de l'ulcère, le recours à la chirurgie en plus d'une antibiothérapie est indispensable, entraînant un lourd impact socio-économique (19).

En pays endémique, le diagnostic est avant tout clinique. Cependant, dans de nombreux pays notamment d'Afrique, l'ulcère de Buruli est souvent co-endémique avec d'autres maladies tropicales négligées à manifestations cutanées et avec des plaies chroniques et cancéreuses, qui présentent des signes cliniques similaires (12). Aussi, est-il nécessaire de confirmer les cas suspects par des examens paracliniques. La confirmation du diagnostic clinique est la PCR quantitative ciblant la séquence IS2404, présente en plus de 200 exemplaires sur le génome de la bactérie. Elle s'avère être le plus sensible et le plus spécifique des tests établis pour la détection de *M. ulcerans*, elle est donc actuellement considérée comme le *gold standard* par l'OMS (4).

Le Bénin est depuis de nombreuses années un pays endémique pour l'ulcère de Buruli (25). Ce sont les CDTLUB qui sont impliqués dans la prise en charge de cette maladie. En novembre 2012, un laboratoire de biologie moléculaire de diagnostic de l'ulcère de Buruli a été

installé par notre équipe de recherche ATOMycA dans le CDTLUB de Pobè, au sud du Bénin, et les techniciens ont été formés à la réalisation de la qPCR (26).

Depuis sa création, le laboratoire de Pobè a montré une évolution importante dans le domaine du diagnostic moléculaire d'autres MTN-C et a participé à différents programmes et réseaux de laboratoires dans le but de lutter contre ces maladies (27).

Le but de **l'article 1** a été de faire le point sur les 10 premières années d'activité de diagnostic de l'ulcère de Buruli du laboratoire de biologie moléculaire du CDTLUB de Pobè et de décrire l'évolution de ce laboratoire pour devenir un laboratoire de référence dans le domaine du diagnostic moléculaire.

Au Bénin, d'autres MTN-C sont endémiques en plus de l'ulcère de Buruli. La prise en charge de ces maladies est rendue difficile du fait de l'éloignement des personnes infectées, vivant dans des régions rurales avec peu ou pas d'accès aux structures de soins. Sur la base de ces observations, des consultations foraines, utilisant des équipes mobiles pour le dépistage intégré des maladies de la peau dont les MTN-C, ont été mises en place dans les départements de l'Ouémé et du Plateau au sud du Bénin afin de diminuer le fardeau de ces maladies.

Le but de **l'article 2** est présenter les résultats de ces consultations foraines réalisées en 2018, 2019 et 2020 et de partager les leçons apprises lors de cette expérience de prise en charge intégrée des MTN-C dans les communes rurales du Bénin.

Article 1: An overview of 10 years of activity of a molecular laboratory for Buruli ulcer diagnosis at a field hospital in Benin.


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An Overview of 10 Years of Activity of a Molecular Laboratory for Buruli Ulcer Diagnosis at a Field Hospital in Benin

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ABSTRACT Buruli ulcer (BU) is a neglected tropical disease caused by *Mycobacterium ulcerans*. Early diagnosis is crucial to prevent morbidity. In November 2012, a field laboratory fully equipped for the rapid on-site quantitative PCR (qPCR) diagnosis of *M. ulcerans* was established at the Buruli ulcer treatment center (CDTLUB) center in Pobè Benin, a region where BU is endemic. We describe its first 10 years of activity and its gradual evolution into an expert laboratory for BU diagnosis. From 2012 to 2022, the laboratory analyzed 3,018 samples from patients attending consultations for suspected BU at the CDTLUB in Pobè. Ziehl-Neelsen staining and qPCR targeting the IS2404 sequence were performed. Since 2019, the laboratory has also received and analyzed 570 samples from other centers. The laboratory confirmed the diagnosis of BU by qPCR for 39.7% samples: *M. ulcerans* DNA was detected in 34.7% of swabs, 47.2% of all fine needle aspiration samples (FNA) and 44.6% of all skin biopsy specimens. Positive Ziehl-Neelsen staining results were obtained for 19.0% samples. Bacterial load, estimated by qPCR, was significantly greater for the Ziehl-Neelsen-positive samples than for Ziehl-Neelsen-negative samples, and detection rates were highest for FNA samples. Overall, 26.3% of the samples received from other centers were positive for BU. Most of these samples were sent by the CDTLUBs of Lalo, Allada, and Zagnanado, Benin. The establishment of the laboratory in the CDTLUB of Pobè has been a huge success. Optimal patient care depends on the close proximity of a molecular biology structure to BU treatment centers. Finally, FNA should be promoted among caregivers.

IMPORTANCE Here, we describe the first 10 years of activity at a field laboratory established at the Buruli ulcer treatment center (CDTLUB) in Pobè, Benin, a country in which *Mycobacterium ulcerans* is endemic. Between 2012 and 2022, the laboratory analyzed 3,018 samples from patients consulting the CDTLUB of Pobè with a suspected clinical BU. Ziehl-Neelsen staining and qPCR targeting the IS2404 sequence were performed. In total, 39.7% of samples tested positive by qPCR and 19.0% tested positive by Ziehl-Neelsen staining. Detection rates were highest for FNA samples, and the bacterial loads estimated by qPCR were significantly higher for Ziehl-Neelsen-positive samples than for

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Ziehl-Neelsen-negative samples. Since 2019, the laboratory has also analyzed 570 samples received from outside the CDTLUB of Pobè, 26.3% of which were positive for BU. Most of these samples were sent by the CDTLUBs of Lalo, Allada, and Zagnanado in Benin. The establishment of the laboratory in the CDTLUB of Pobè has been a huge success, with major benefits for both the medical staff and patients. Our findings illustrate that the usefulness and feasibility of having a diagnostic center in rural Africa, where the disease is endemic, is a key part of optimal patient care, and that FNA should be promoted to increase detection rates.

KEYWORDS Buruli ulcer, PCR, molecular biology laboratory, Benin, *Mycobacterium ulcerans*, diagnosis

Neglected tropical diseases (NTDs) are poverty-related and affect almost two billion people, mostly living in tropical and subtropical regions in marginalized communities in Africa, Asia, and the Americas (1). Buruli ulcer (BU) is one such disease. It has cutaneous manifestations and is caused by *Mycobacterium ulcerans*, a pathogenic environmental mycobacterium that produces a toxin called mycolactone, which has cytotoxic, immunomodulatory, and analgesic properties, leading to severe but painless skin ulcerations (2). Clinically, BU begins with a nodule, papule, edematous lesion, or plaque which frequently progresses to extensive skin ulceration; in the absence of treatment, this can lead to malformations and permanent disability, requiring long periods of hospitalization and surgical interventions that may be prohibitively expensive for poor families. Early diagnosis and treatment initiation are, therefore, essential, to prevent the deleterious consequences of this disease (3, 4).

Two methods are currently used in the field to confirm a clinical diagnosis of BU: Ziehl-Neelsen staining to detect acid-fast bacilli and qPCR for the detection of pathogen-specific DNA (5–8). Quantitative PCR for the IS2404 sequence has proven the most sensitive and specific of the established tests for detecting *M. ulcerans*, and is therefore currently considered the gold standard for the confirmation of clinical diagnosis (5, 9, 10). The WHO recommends PCR confirmation before the start of treatment to rule out differential diagnoses because this approach can facilitate identification of patients with non-BU chronic ulcers (8). Sampling techniques depend on the nature of the lesions. Fine needle aspiration (FNA) is performed on non-ulcerative lesions (plaques, nodules, and edemas), whereas swabs are taken from under the edges of ulcerative lesions (6, 7). Finally, excised necrotic tissue can be sent to the laboratory, but this sampling method is no longer recommended.

In Africa, BU is common in the central and western regions, particularly Côte d'Ivoire, Ghana, Nigeria, and Benin (11, 12). In 2004, a Buruli ulcer diagnosis and treatment center (Centre de Diagnostic et de Traitement de la Lèpre et de l'Ulcère de Buruli [CDTLUB]) was constructed in Pobè, a city in the Plateau area of Benin known to have a high incidence of *M. ulcerans* infections. In November 2012, a new laboratory for BU diagnosis by qPCR was established, and its technicians were trained to perform this technique (13). During the first few years, qPCR was systematically performed in duplicate, in the CDTLUB laboratory and the bacteriology laboratory of Angers University Hospital, France, to check the quality of the analysis. The results obtained were good, and, since 2014, the qPCR has been performed at the CDTLUB laboratory in Benin only. Since its creation, this laboratory has participated in different available external quality assessment programs (5, 14). Here, we provide a report of the first 10 years of *M. ulcerans* diagnostic activities at the CDTLUB laboratory, together with our drawn conclusions and recommendations.

MATERIALS AND METHODS

Between 2012 and 2022, the CDTLUB laboratory received and analyzed diverse samples consisting of swabs, FNA, and skin biopsy specimens. The laboratory received dried swabs and biopsy specimens or FNA resuspended in water, always transported in an icebox, as recommended by the Standard Operating Protocols of the BU-LabNet (5). Each sample underwent Ziehl-Neelsen staining and qPCR for the IS2404 sequence. Sample preparation and Ziehl-Neelsen staining were performed as previously described (13). Swabs were rehydrated and biopsy specimens were minced in 2 mL of sterile water. From 2012 to 2019, DNA extraction procedures and qPCR were performed as previously described (13). For DNA preparation, 400 μ L of sample suspension was centrifuged, and the pellet was resuspended in 50 mM NaOH and heated at 95°C for

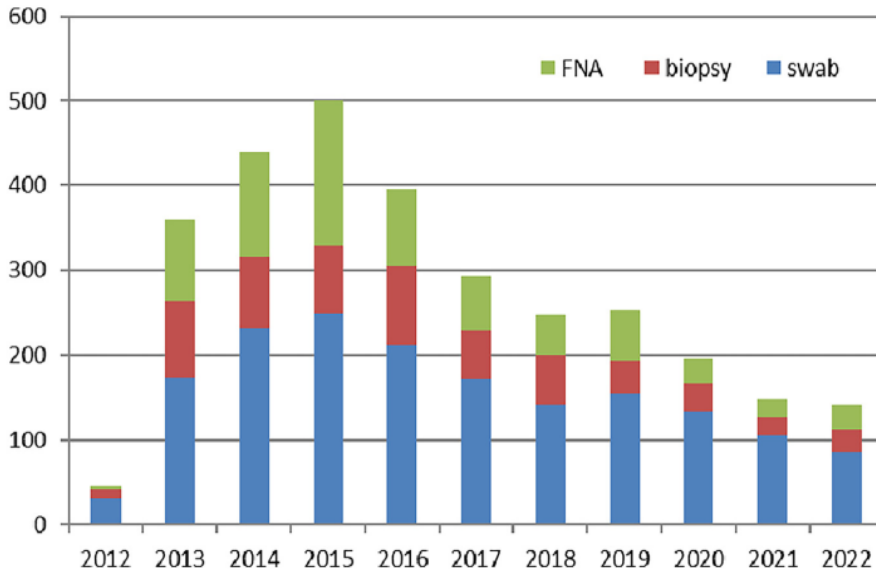


FIG 1 Changes in the numbers of samples from the Buruli ulcer treatment center (CDTLUB) of Pobè, Benin, analyzed by the laboratory between 2012 and 2022 for the detection of *Mycobacterium ulcerans*.

10 min. DNA was then isolated using the QIAquick PCR purification kit (Qiagen, Hilden, Germany), in accordance with the manufacturer’s instructions. Negative and positive controls were systematically included. Sample DNA was quantified with an external standard curve constructed with a series of six 10-fold serial dilutions of *M. ulcerans* (strain 1G897) DNA. Since January 2020, the CDTLUB laboratory of Pobè has been part of the BU-LabNet network and, as such, follows its standard operating procedures for DNA extraction and purification and qPCR. DNA is extracted using the Genolyse kit (Hain LifeScience GmbH, Nehren, Germany), which uses a similar method to the previous one based on an alkaline lysis and heat. Also, quantification is performed using a plasmid instead of purified *M. ulcerans* DNA (5).

No ethics clearance was required for this retrospective laboratory study.

RESULTS

Analysis of samples from patients consulting at the CDTLUB of Pobè. Between 2012 and 2022, 3,018 samples collected from patients consulting at the CDTLUB of Pobè were analyzed by the laboratory: 1,686 samples (55.9%) were swabs, 742 (24.6%) were FNA samples, and 590 (19.5%) were skin biopsy specimens. The number of analyses performed increased from 2012, reaching 501 samples in 2015. The number of samples analyzed by the laboratory at the CDTLUB of Pobè subsequently decreased to below 200 (Fig. 1). Over the 10 years of activity at this laboratory, swabs were the most frequently tested sample type, accounting for 48.1% (in 2013) to 71.9% (in 2020) of samples. FNA samples accounted for 8.9% of all samples analyzed in 2012, increasing to 34.5% in 2015. Finally, biopsy specimens from excised tissues accounted for 24.4% of analyzed samples in 2012, but only 13.0% in 2021.

In total, 1,198 (39.7%) samples tested positive for *M. ulcerans* by qPCR. DNA was detected in 585/1,686 swabs (34.7%), 350/742 FNA samples (47.2%), and 263/590 skin biopsy samples (44.6%) (Table 1, Fig. 2). Ziehl-Neelsen staining was positive for 574 samples (19.0%), with positivity rates highest for biopsy specimens (25.6%), followed by swab samples (19.3%) and then FNA samples (13.2%) (Table 1). Bacterial loads, as estimated by qPCR, differed between sample types. Skin biopsy specimens contained the

TABLE 1 Comparison between qPCR and Ziehl-Neelsen staining results for the detection of *Mycobacterium ulcerans* in swabs, FNA samples, and biopsy specimens^a

| Sample | Swab | FNA | Biopsy specimen | Total |
|--|-----------------------|-----------------------|-----------------------|-----------------------|
| Total (2012–2022) | 1,686 | 742 | 590 | 3,018 |
| qPCR-positive, n (%) | 585 (34.7) | 350 (47.2) | 263 (44.6) | 1,198 (39.7) |
| Mean bacterial DNA load estimated by qPCR for positive samples (genome units/mL) | 5.4 × 10 ⁶ | 5.7 × 10 ⁶ | 2.2 × 10 ⁷ | 9.2 × 10 ⁶ |
| Ziehl-Neelsen-positive, n (%) | 325 (19.3) | 98 (13.2) | 151 (25.6) | 574 (19.0) |

^aFNA, fine needle aspiration.

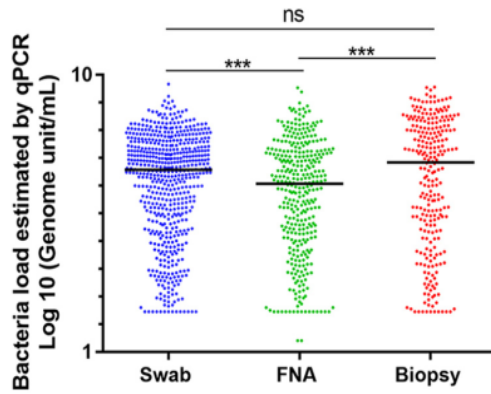


FIG 2 Quantification of *M. ulcerans* DNA by quantitative PCR (qPCR) analysis on swabs, fine needle aspiration samples, and biopsy specimens ($n = 3,018$). ***, $P < 0.001$; ns, not significant. Bacterial load was assessed by qPCR and is expressed as the number of genome units per mL.

largest amounts of *M. ulcerans* DNA, with a mean load of 2.2×10^7 genome units (GU/mL), followed by FNA samples (5.7×10^6 GU/mL) and then swabs (5.4×10^6 GU/mL). The mean load was significantly higher for biopsy specimens than for FNA samples ($P < 0.001$, Cohen's $d = 0.0042$) and swabs ($P < 0.001$, Cohen's $d = 0.20$) (Fig. 2). Bacterial load, as estimated by qPCR, was significantly greater for Ziehl-positive than for Ziehl-negative samples ($P < 0.0001$, Cohen's $d = 0.23$) (Fig. 3A and B).

Analysis of samples from patients consulting outside the CDTLUB of Pobè. From 2019 onwards, the laboratory analyzed a total of 570 samples from patients consulting outside the CDTLUB of Pobè. These samples included 336 from the CDTLUB of Lalo, 123 from the CDTLUB of Zagnanado, and 92 from the CDTLUB of Allada, in Benin (Table 2). These samples accounted for 96.7% of the external samples analyzed. Swabs accounted for 80.4% of samples from Allada, whereas FNA samples accounted for 19.6%. No biopsy specimens were received from Allada. Most (94.9%) of the samples from Lalo were swabs, but 5.1% were FNA samples. The samples from Zagnanado addressed to the CDTLUB of Pobè were either swabs (58.5%) or skin biopsy specimens (41.5%). Positivity rates were highest for the samples from Allada (59.8%), followed by those from Zagnanado (23.6%) and then those from Lalo (19.3%) (Table 2). The other external samples, 13 from Tchaourou Goro, 1 from Cotonou University Hospital, and 1 from Parakou, all tested negative. Finally, the CDTLUB laboratory in Pobè received three samples from Sierra Leone, all of which tested negative, and one sample from Congo-Brazzaville, which tested positive for BU by qPCR (Table 2).

Time from sample reception to results. PCR test results must be delivered as rapidly as possible if they are to be useful to clinicians and help improve patient management. We assessed the testing turnaround times using two parameters for the year 2021: time (in days) from reception of the sample at the laboratory to the delivery of PCR results, and time

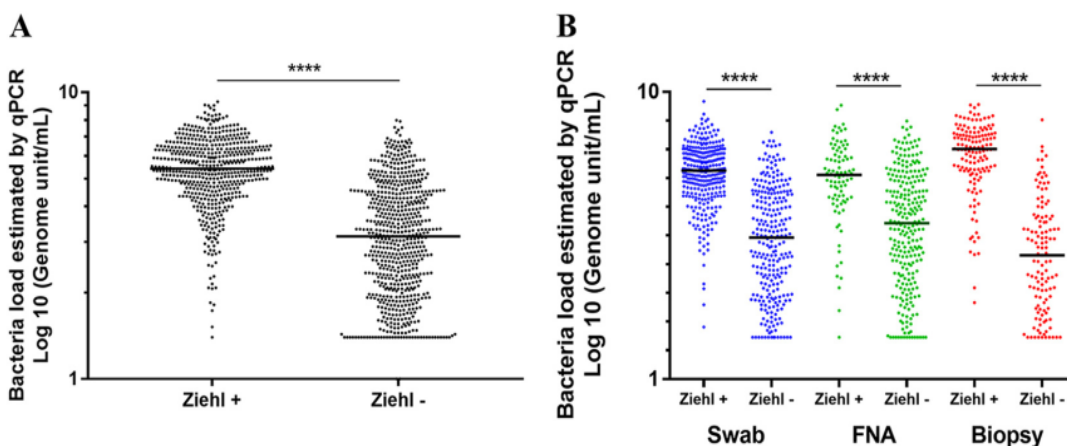


FIG 3 Quantification of *M. ulcerans* DNA by qPCR by (A) positivity or negativity for Ziehl-Neelsen staining and by (B) sample type and Ziehl-Neelsen staining results. Bacterial load was determined by qPCR and is expressed as the number of genome units per mL. ****, $P < 0.0001$. FNA, fine needle aspiration.

TABLE 2 *Mycobacterium ulcerans* detection results for samples from patients consulting outside the CDTLUB of Pobè^a

| Region | Swab | | FNA | | Biopsy specimen | | Total | |
|-------------------|-------------------|----------------------------------|-------------------|----------------------------------|-------------------|----------------------------------|-------------------|----------------------------------|
| | Samples, <i>n</i> | qPCR ⁺ , <i>n</i> (%) | Samples, <i>n</i> | qPCR ⁺ , <i>n</i> (%) | Samples, <i>n</i> | qPCR ⁺ , <i>n</i> (%) | Samples, <i>n</i> | qPCR ⁺ , <i>n</i> (%) |
| Allada | 74 | 46 (62.2) | 18 | 9 (50.0) | 0 | NA | 92 | 55 (59.8) |
| Lalo | 319 | 59 (18.5) | 17 | 6 (35.3) | 0 | NA | 336 | 65 (19.3) |
| Zagnanado | 72 | 8 (11.1) | 0 | NA ^b | 51 | 21 (41.2) | 123 | 29 (23.6) |
| Tchaorou Goro | 13 | 0 (0) | 0 | NA | 0 | NA | 13 | 0 (0) |
| CNHU Cotonou | 1 | 0 (0) | 0 | NA | 0 | NA | 1 | 0 (0) |
| PNLUB/Parakou | 1 | 0 (0) | 0 | NA | 0 | NA | 1 | 0 (0) |
| Congo-Brazzaville | 1 | 1 (100) | 0 | NA | 0 | NA | 1 | 1 (100) |
| Sierra Leone | 2 | 0 (0) | 1 | 0 (0) | 0 | NA | 3 | 0 (0) |

^aCDTLUB, Buruli ulcer treatment center; qPCR, quantitative PCR; CNHU Cotonou, Cotonou University Hospital; PNLUB, Programme National de Lutte contre L'Ulcère de Buruli; FNA, fine needle aspiration.

^bNA, not applicable.

from sample collection from the patient to sample delivery to the laboratory (Fig. 4). We differentiated between samples obtained directly from patients consulting at the CDTLUB of Pobè and samples sent by the other CDTLUBs in Benin. It should be noted that of the 4 CDTLUBs in Benin, only the Pobè center is equipped with a molecular biology laboratory. The median time to PCR results after sample reception was 2 days (interquartile range [IQR]: 0 to 4 days) for samples from the CDTLUB of Pobè and 1 day (IQR: 1 to 2 days) for external samples. As expected, samples were taken and delivered to the laboratory on the same day (median interval: 0 days; IQR: 0 to 2 days) for samples obtained from the CDTLUB of Pobè. However, the time from sample collection to sample delivery to the laboratory was more significant for the samples from the other CDTLUBs (median: 15 days; IQR: 7 to 46.5 days).

DISCUSSION

Several key findings emerge from our study of the first 10 years of activity at this laboratory. First, our results show that only 19.0% of samples tested positive by Ziehl-Neelsen staining, whereas 39.7% tested positive for *M. ulcerans* by qPCR. These observations confirm the choice of qPCR as the gold standard for the confirmation of clinical diagnoses of BU. Our findings also highlight the importance of setting up laboratories for rapid and reliable qPCR testing in areas where a disease is endemic, close to the patients. Second, swabs were the most frequently analyzed sample type in our study. However, PCR positivity rates were highest for FNA samples, even though these samples gave lower rates of positivity for Ziehl-Neelsen

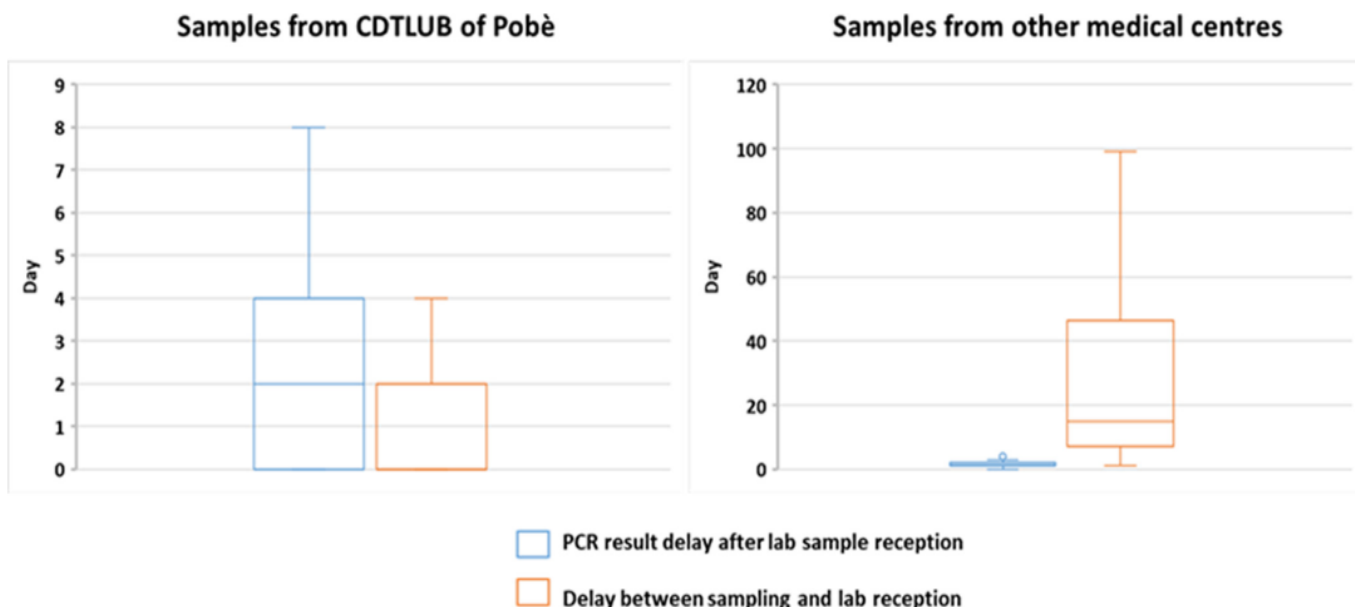


FIG 4 Time to PCR results. Median number of days from sample reception by the laboratory to the delivery of PCR results (blue) and median number of days from patient sampling to reception of the sample by the laboratory (orange). Left: samples from the CDTLUB of Pobè. Right: samples from other CDTLUBs in Benin.

staining than swabs and skin biopsy specimens. Our findings also confirm the results of our previous studies (6, 13). FNA is generally recommended for closed lesions but can also be performed on ulcerated forms with induration because large numbers of bacilli can be retrieved from these areas. The number of FNA samples analyzed at the CDTLUB of Pobè increased rapidly following the introduction of qPCR testing at this laboratory, but has tended to decrease in recent years. Furthermore, little or no FNA-based sampling is performed at the other CDTLUBs. We can only encourage the more frequent use of FNA instead of, or in addition to, swab sampling. The feasibility of FNA and the availability of consumables for this technique are similar to those for swabbing in Benin. In addition, it has been shown that FNA is less painful than swabbing (6). Given the higher detection rates achieved with FNA, we should therefore encourage the use of FNA for biological sampling in patients with suspected Buruli ulcer.

We noted a decrease in the number of tests performed by the CDTLUB of Pobè from 2015 onwards. These observations may partly account for the decrease in new Buruli ulcer cases in Benin reported since 2012 (15, 16). Since its creation a decade ago, the laboratory of the CDTLUB in Pobè has achieved recognition as a reference laboratory for the diagnosis and analysis of samples from patients with suspected BU. This has led to the center not only performing its own testing autonomously, but also to receiving samples from other CDTLUBs in Benin, with logistic support from the PNLLUB (the national program for Buruli ulcer and leprosy), the Raoul Follereau Foundation, and the Anesvad Foundation. The number of samples received from outside the CDTLUB of Pobè is increasing, probably because the results are obtained very quickly (a median of 1 day after sample reception). Positivity rates were found to be higher for samples from the CDTLUB of Allada than for samples from the other CDTLUBs. The principal reason for this is probably the use of WHO clinical diagnosis scoring criteria by the medical staff of Allada (17), leading to the collection of samples only from patients for whom these criteria indicated the diagnosis to be “very likely BU” or “likely BU.” The other CDTLUBs systematically sample cutaneous ulcers without using this score. Harmonization of the criteria for selecting patients with this scoring grid for biological confirmation is the next objective for the Beninese National Program for Buruli Ulcer (PNLLUB).

Another demonstration of the expertise acquired by the CDTLUB laboratory is its participation as a founding member of the BU-LabNet program, a new model program supervised by the WHO and consisting of a network of 11 laboratories located in African countries where BU is the most endemic, for external quality assessments of PCR-based molecular diagnosis (5). This program supplies all the reagents required for DNA extraction and qPCR amplification free of charge, once annually.

Building on the molecular expertise acquired by the laboratory doctors and technicians following the establishment of qPCR testing for BU diagnosis, an important evolution was observed in 2018 with the implementation of the RLEP-qPCR targeting *Mycobacterium leprae* DNA specific sequence (18, 19). Currently, during 2023, the aim is to establish GenoType LepaeDR testing (Hain Lifescience GmbH, Nehren, Germany) for the detection of genes conferring resistance to rifampicin, ofloxacin, and dapsone (20); optimize the adaptation of antibiotic treatment; and participate in the surveillance of antimicrobial resistance in leprosy. Finally, following the outbreak of the COVID-19 pandemic in 2020, the CDTLUB laboratory rapidly implemented PCR tests for SARS-CoV-2 at the request of the Health Ministry of Benin. Between April 2020 and August 2021, more than 30,000 PCR tests for SARS-CoV-2 were performed by this laboratory for COVID diagnosis.

Conclusion. The establishment of the CDTLUB laboratory in Pobè has been a huge success. The location of this laboratory in a zone in which BU is endemic has been advantageous for both the medical staff and patients. The expertise and dynamism of both the doctors and technicians have enabled the laboratory to invest in other diagnostic missions in addition to that for which it was originally established.

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All authors have read and approved the manuscript.

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The authors have no competing interests to declare.

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Article 2: Importance of consultations using mobile teams in the screening and treatment of neglected tropical skin diseases in Benin.

Gnimavo RS*, **Fajloun F***, Al-Bayssari C, Sodjinou E, Habib A, Ganlonon L, Claco E, Agoundoté I, Houngbo OA, Anagonou EG, Biaou CAO, Ayélo AG, Houezo JG, Boccarossa A, Moussa EH, Gomez B, Gine A, Sopoh GE, Marion E, Johnson RC, Kempf M.

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RESEARCH ARTICLE

Importance of consultations using mobile teams in the screening and treatment of neglected tropical skin diseases in Benin

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Abstract

Context

Since 2013, the World Health Organization has recommended integrated control strategies for neglected tropical diseases (NTDs) with skin manifestations. We evaluated the implementation of an integrated approach to the early detection and rapid treatment of skin NTDs based on mobile clinics in the Ouémé and Plateau areas of Benin.

Methods

This descriptive cross-sectional study was performed in Ouémé and Plateau in Benin from 2018 to 2020. Consultations using mobile teams were performed at various sites selected by reasoned choice based on the epidemiological data of the National Program for the Control of Leprosy and Buruli Ulcer. All individuals presenting with a dermatological lesion who voluntarily approached the multidisciplinary management team on the day of consultation were included. The information collected was kept strictly anonymous and was entered into an Excel 2013 spreadsheet and analyzed with Stata 11 software.

Results

In total, 5,267 patients with various skin conditions consulted the medical team. The median age of these patients was 14 years (IQR: 7–34 years). We saw 646 (12.3%) patients

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presenting NTDs with skin manifestations, principally scabies, in 88.4% (571/646), followed by 37 cases of Buruli ulcer (5.8%), 22 cases of leprosy (3.4%), 15 cases of lymphatic filariasis (2.3%) and one case of mycetoma (0.2%). We detected no cases of yaws.

Conclusion

This sustainable approach could help to decrease the burden of skin NTDs in resource-limited countries.

Author summary

Neglected Tropical Diseases (NTDs) with skin manifestations are a group of nine infectious diseases with skin manifestations, either as the primary symptom or as an associated clinical feature. They are often co-endemic and harbor similar clinical signs.

We evaluated the implementation of an integrated approach to the early detection and rapid treatment of skin NTDs based on consultations using mobile teams in the *départements* of Ouémé and Plateau in Benin.

In total, 5,267 patients with various skin conditions consulted. We saw 646 (12.3%) patients presenting NTDs with skin manifestations and among them, 571 (88.4%) had scabies, 37 (5.8%) had Buruli ulcer, 22 (3.4%) had leprosy, 15 (2.3%) had lymphatic filariasis and 1 (0.2%) had mycetoma.

Consultations using mobile teams constitute a very efficient strategy for the integrated screening of skin diseases and especially of NTDs with skin manifestations. This sustainable approach could help to decrease the burden of NTDs in resource-limited countries.

I. Introduction

Neglected tropical diseases (NTDs) constitute a group of 20 prevalent infectious diseases affecting more than a billion people worldwide. They are particularly prevalent in poor countries with a tropical or subtropical climate, particularly in isolated rural areas, conflict zones and informal settlements. They are very common in areas without a safe supply of drinking water and with poor access to healthcare facilities, where people live in poor hygiene conditions, close to animals and the vectors of infectious disease [1–3]. NTDs cost billions of dollars in terms of healthcare costs and lost productivity, providing a strong economic argument for investment in their prevention, treatment and cure [4–5].

Nine of the 20 NTDs have skin manifestations, either as the primary symptom or as an associated clinical feature [6]. These diseases are Buruli ulcer, leprosy, yaws, scabies, lymphatic filariasis, mycetoma, cutaneous leishmaniasis, post-Kala azar dermal leishmaniasis and onchocerciasis [7]. Unfortunately, these conditions are often diagnosed late, in patients with visible skin deformations or physical disabilities, a poor functional prognosis and alterations to quality of life. The victims of these skin NTDs are subjected to societal stigmatization throughout their lives, potentially resulting in total destitution, requiring them to beg, and significant psychological damage [8,9].

Skin NTDs have long been endemic in West African countries, including Nigeria, Benin, Ghana, Côte d'Ivoire, Burkina Faso and Togo in particular, and encouraging progress has been made towards combating them [10–12]. The World Health Assembly, held in May 2013, and the World Health Organization (WHO) Regional Committee for Africa, held in

September of the same year, both advocated the creation of NTD management programs [13,14]. The following year, the WHO African Region published its regional strategy for NTDs, in which one of the targets identified was the elimination of leprosy, control of Buruli ulcer and eradication of yaws. As a means of attaining this objective, WHO African Region requested the implementation of national NTD plans and integrated NTD programs at national level in all countries in which these diseases were endemic [10, 11].

Skin NTDs are common in Benin, a country in which both Buruli ulcer and leprosy are endemic [4]. Most of the other skin NTDs are also frequently detected in Benin [15,16]. Coordination to combat these conditions was established in 2017 in Benin. Efforts were coordinated by the National Program for the Control of Leprosy and Buruli Ulcer (PNLLUB), composed of Buruli ulcer treatment Centers (CDTLUB—*Centre de Dépistage et de Traitement de la Lèpre et de l'Ulcère de Buruli*) and Leprosy Treatment Centers (CTAL—*Centre de Traitement Anti-Lèpre*). This system relies on nursing supervisors and community relays working in collaboration with the CDTLUBs and CTALs.

This national health program has established a surveillance system for the detection, notification and management of cases by several diagnosis and treatment centers in each *département* (an administrative area equivalent to a county) of the country. The epidemiological data from this surveillance system revealed high detection rates of Buruli ulcer and leprosy in the *départements* of Ouémé and Plateau [17,18]. Based on these observations, consultations using mobile teams (mobile consultations) for the integrated screening of skin diseases, including skin NTDs in particular, were performed in these areas to decrease the burden of morbidity. These consultations made it possible to screen people and to detect skin diseases, including NTDs, earlier, and to provide treatment to prevent progression and deleterious complications.

The goal of this study is to share the lessons learned during this experience of integrated management of skin NTDs in rural districts of Benin. We report here the results of this integrated approach involving mobile consultations in Ouémé and Plateau in Benin, in 2018, 2019 and 2020.

II. Framework and study methods

2.1. Implementation framework

We used an approach based on free mobile clinics in the *départements* of Ouémé and Plateau in Benin. This approach was initiated jointly by the National Leprosy Control Program and the Raoul Follereau Foundation of France. A multidisciplinary medical team (nurses, dermatology/leprosy nurses, a biomedical engineer, general practitioner, dermatologist, and public health specialist) from the CDTLUB in Pobè was responsible for performing these consultations under the supervision of PNLLUB managers.

2.2. Study Methods

Ethics approval and consent for participation. Authorization for data collection for this study was obtained from the National Health Research Ethics Committee (Reference no. 21/MS/DC/SGM/DRFMT/CNERS/SA). These mobile consultations were part of a broader study of the factors contributing to the performance of primary and secondary prevention activities for skin NTDs in Benin covered by ethics committee authorization no. 0478/CLERB/UP/P/SP/R/SA.

On site, the objectives of the survey were clearly explained to the participants and the questionnaires were administered only free and informed verbal consent had been obtained. Confidentiality was guaranteed for the information provided, which was collected solely for the purposes of the study and will remain anonymous. We confirm that all the methods used in

this observational study complied with all research guidelines and regulations in force. All the experimental protocols involving humans described here were performed in accordance with national/international/institutional guidelines and the Declaration of Helsinki. For the children enrolled in this study, free verbal consent was obtained from their parents or guardians.

2.2.1. Preparatory phase. The preparatory phase involved writing letters to political and administrative authorities to explain the context, objectives and the expected results for this activity. We then trained health workers (head nurses from peripheral healthcare facilities, laboratory technicians), community relays, village volunteers and teachers. This integrated training focused on basic epidemiology, elements of positive and differential diagnosis, complications, principles of care and strategies for fighting the principal neglected tropical diseases with skin manifestations found in these two areas (Buruli ulcer, leprosy, yaws) according to data from the National Program for the Fight against Leprosy and Buruli Ulcer of Benin.

Religious leaders, local elected officials, rulers, town criers, megaphonists and village chiefs from the various sites were also briefed about these diseases to raise their awareness, in discussion sessions focusing on the epidemiology of the disease, the signs and symptoms useful for early diagnosis and, in particular, the availability of free treatment for patients suffering from these diseases attending the mobile clinics.

Social mobilization was ensured by town criers and megaphonists charged with the mission of informing the community before the arrival of the medical team and on the day of the activity.

Radio broadcasts also went out in local languages on community radio stations (*Le Roi de la Vallée* in Ouémé, and *Olokiki* and *Alakétou* in Plateau) to raise listeners' awareness about the existence of these diseases, their signs and early symptoms, the availability of free treatment and the timetable for the series of mobile consultations scheduled for the population, whose well-being was the priority at the heart of the initiative. This primordial step made it possible to inform the community, to prepare the community for this initiative so as to obtain its agreement, commitment, adherence, involvement and active participation, to ensure the success of this intervention.

2.2.2. Implementation of the phase of activity. This cross-sectional, descriptive study that took place in the *départements* of Ouémé and Plateau in Benin in 2018 (November 6 to December 20), 2019 (May 2 to August 17) and 2020 (December 1 to 22). The primary target population consisted of all individuals with dermatological lesions residing in Ouémé and Plateau during the period of in which this intervention took place.

All individuals with a dermatological lesion voluntarily presenting for consultation and examination by the multidisciplinary medical management team on the day of the consultation from whom free verbal consent for treatment was obtained were included. Those who refused to be seen were not included.

2.2.3. Sampling methods and techniques. Given the limited resources allocated to the fight against skin NTDs and based on the epidemiological data available from the National Leprosy and Buruli Ulcer Control Program of Benin [19], we used a non-probabilistic reasoned-choice method to select the sites for the implementation of these consultations in Ouémé and Plateau.

Finally, we used a technique of choice by convenience to select all individuals presenting dermatological lesions presenting themselves voluntarily to the multidisciplinary management team on the day of the consultation. All these people were carefully examined in a room lit by daylight, in strict respect of human dignity and in accordance with the requirements of the medical ethics code in force in the Benin [20]. Samples were collected from all suspected cases of Buruli ulcer and yaws, after verbal consent for biological confirmation had been obtained from the patient. In cases of suspected BU, swabs, taken from the undermined edges of the

ulcer, or fine-needle aspiration samples were collected and sent to the CDTLUB laboratory in Pobe for biological confirmation by polymerase chain reaction (PCR) for IS2404 [21,22]. For the confirmation of yaws, samples from suspected cases were subjected to a non-treponemal and treponemal rapid diagnostic test (DPP Syphilis Screen and Confirm Assay, Chembio Diagnostic Systems, Medford, NY, USA). Leprosy was diagnosed essentially on clinical grounds, in accordance with the recommendations of the WHO Expert Committees on Leprosy [23].

In the absence of video dermatoscopy, a diagnosis of scabies was retained on the basis of the alternative level B consensus criteria for the clinical diagnosis of scabies diagnosis of the International Alliance for the Control of Scabies [24].

2.2.4. Study tools and variables. An integrated data form was used to collect information on skin NTDs. On this form, we recorded sociodemographic data (age, sex, main occupation or profession of the respondent, level of education, ethnicity, religion, name of the village, district and community of origin of the patient) together with geographic coordinates and information about the socioeconomic status of the respondents. This last parameter was according to the principal component analysis (PCA) method described by Filmer et al [25].

The clinical characteristics of the dermatological lesions, the nature of the tests performed and of the samples taken, the results of the tests, the diagnosis attributed and the course of action of the management team were also recorded.

2.2.5. Data processing and analysis. After data collection, the forms were manually processed to check the completeness and consistency of the data collected. A careful review and sorting of the data was also done to look for duplicates. All of these steps allowed us to reassure of the quality of the collected data. In the absence of any inconsistencies, the data were entered into an Excel 2013 spreadsheet and analyzed with Stata 11 software.

The variables obtained included descriptions of the epidemiological, clinical and biological characteristics of cases. Proportions were calculated for the qualitative variables. Quantitative variables are expressed as means, with the standard deviation also indicated for those following a normal distribution. The median and range were determined for quantitative variables with a skewed distribution.

2.2.6. Mapping. Maps were generated with ArcView GIS 3.2 software. For each confirmed case of skin NTDs, the geographic coordinates were noted and projected onto the background map downloaded free of charge from the DIVA GIS website (<https://www.diva-gis.org/>). Datas for the administrative boundary were provided by Ministry of Territory Planning of Benin (https://gadm.org/download_country_v3.html).

III. Results

3.1. General characteristics of the individuals with skin diseases

In total, 5,267 people consulted for skin diseases at the mobile clinics during the three study periods (Fig 1). During the first period (November 6 to December 20, 2018), 607 people were seen in Ouémé and Plateau. During the second period (May 2 to Aug 17, 2019), we saw 2,444 people in the two *départements*. During the third period (Dec 1 to Dec 21, 2020) 2,216 people were seen, exclusively in Plateau. The various administrative areas and districts visited by the mobile consultation team during the three periods are presented in Table 1 and Fig 2. The main characteristics of the patients are summarized in Table 1.

3.2. Diagnosis of neglected tropical skin diseases

In total, 648 (12.3%) patients seen at mobile consultations were diagnosed with a skin NTD. Skin NTDs accounted for 6.6% (40/607) of all the skin diseases diagnosed in 2018, 6.1% (138/



Fig 1. Organization of mobile consultations (preparatory and implementation phase) in Ouémé and Plateau in Benin, from 2018 to 2020. **A:** Multidisciplinary team from the CDTLUB of Pobè going on foot (with equipment and consumables carried in the hand and on the head in a marshy and flood-prone region, whose soil and dwellings are located at altitude) to Gnanwizoumin, a village in the Department of Ouémé, Municipality of Bonou, Arrondissement of Daméwogon, which benefited from this activity organized by the multidisciplinary team of the CDTLUB of Pobè, on 06/06/19. **B:** Reception, registration of patients (collection of socio-demographic information), taking of constants by the healthcare teams of the CDTLUB de Pobè in Attanchoukpa, a village in the Plateau department, selected to host the implementation of mobile consultations in the Commune of Kétou, District of Odométa on 08/02/20. **C:** realization by a health worker from the CDTLUB (Biomedical Engineer) of Pobè of rapid antigenic diagnostic tests for screening of yaws in children under 15 years old living in Towé, a locality in the Plateau department located in the commune of Pobè and selected for the organization of fairground consultations on 12/12/2020. **D:** Medical prescription followed by an explanatory session of the skin dermatosis and the medical prescription to a mother whose child has been received and examined by a health worker from the multidisciplinary care team of the CDTLUB of Pobè as part of the fairground consultation organized on 11/12/20 in Houédamé, a village in the Plateau department, located in the commune of Adja-Ouèrè, district of Kpoulou.

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2,244) of those diagnosed in 2019, and 21.2% (470/2,216) of those diagnosed in 2020 (Table 2). The sites of the mobile consultations are presented in Fig 3. Scabies was, by far, the most frequent skin NTD, accounting for 72.5% (29/40), 66.7% (92/138) and 95.7% (450/470) of all cases of skin NTDs in 2018, 2019 and 2020. The other skin NTDs diagnosed were Buruli ulcer, leprosy, lymphatic filariasis and mycetoma.

In total, 37 cases of Buruli ulcer were diagnosed during the three periods of mobile consultations, accounting for 5.7% of the skin NTDs and 0.7% of all skin diseases diagnosed (Fig 4A). All these cases were confirmed by PCR. Characteristics of patients presenting Buruli ulcer are presented in Table 3.

During the first period of mobile consultations, three patients (7.5% of the skin NTD cases) tested positive for Buruli ulcer: two male and one female. All cases were systematically treated

Table 1. Characteristics of the individuals with skin diseases seen in Plateau and Ouémé by the mobile consultation team during the three periods of consultations.

| | Period 1 (Nov 6 to Dec 20, 2018) | Period 2 (May 2 to Aug 17, 2019) | Period 3 (Dec 1 to 21, 2020) |
|---|---|---|---|
| Number of cases of skin diseases identified | 607 | 2,444 | 2,216 |
| Median age (range) | 15 y (1–82) | 16 y (1–95) | 12 y (1–75) |
| Sex ratio (M/F) | 1.003 (304/303) | 0.88 (1,147/1,297) | 0.93 (1,067/1,149) |
| Profession, no. (%) | | | |
| Student/pupil | 261 (43.0) | 862 (35.3) | 1,053 (47.5) |
| Tradesperson | 82 (13.5) | 432 (17.8) | 181 (8.2) |
| Farmer | 79 (13.0) | 384 (15.7) | 271 (12.2) |
| No profession | 74 (12.2) | 354 (14.5) | 274 (12.4) |
| Artisan | 66 (10.9) | 238 (9.7) | 205 (9.3) |
| Household | 35 (5.8) | 139 (5.7) | 197 (8.9) |
| Civil servant | 10 (1.7) | 35 (1.4) | 35 (1.6) |
| Municipality, no. (%) | Pobè 230 (37.9) Kétou 138 (22.7) Adja-Ouèrè 82 (13.5) Ifangni 82 (13.5) Dangbo 73 (12.0) Other 2 (0.3) | Dangbo 1,215 (49.7) Adjohoun 716 (29.3) Bonou 420 (17.2) Adja-Ouèrè 57 (2.3) Other 36 (1.5) | Pobè 1,039 (46.9) Adja-Ouèrè 610 (27.5) Kétou 562 (25.4) Other 5 (0.2) |
| Ethnic group, no. (%) | | | |
| Wémé and related | 37 (6.1) | 2,251 (92.1) | 349 (15.8) |
| Holly | 106 (17.5) | 20 (0.8) | 1,081 (48.8) |
| Nagot and related | 209 (34.4) | 19 (0.8) | 705 (31.8) |
| Fon and related | 255 (42.0) | 127 (5.2) | 80 (3.6) |
| Torinu and related | - | 27 (1.1) | 1 (0) |
| Educational level, no. (%) | | | |
| Educated | 271 (44.7) | 897 (36.7) | 1,088 (49.1) |
| Not educated | 336 (55.4) | 1,547 (63.3) | 1,128 (50.9) |
| Socioeconomic level, no. (%) | | | |
| High | - | 5 (0.2) | 22 (1.0) |
| Medium | 79 (13.0) | 160 (6.6) | 177 (8.0) |
| Low | 528 (87.0) | 2,279 (93.3) | 2,017 (91.0) |

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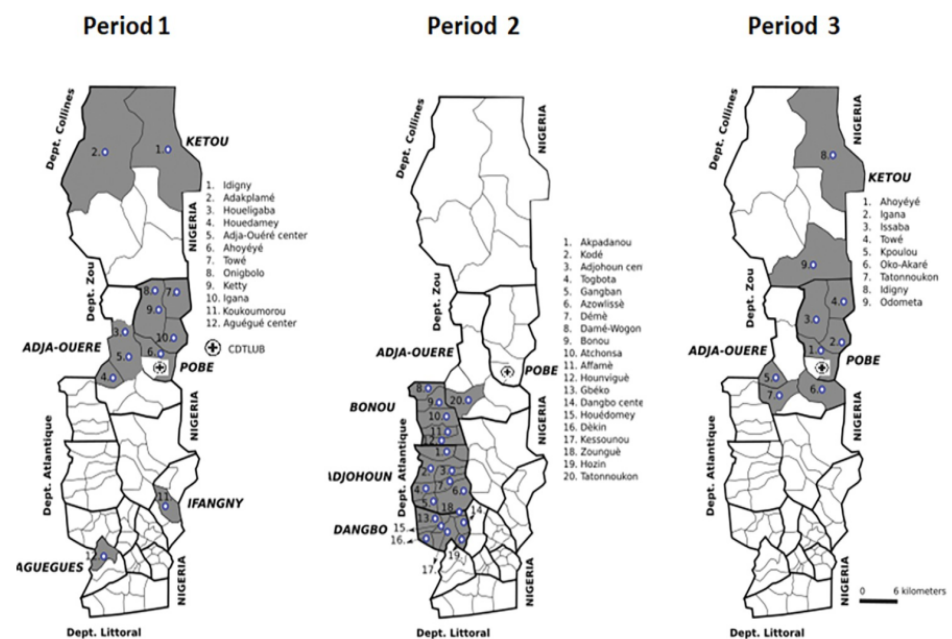


Fig 2. Districts visited during the three periods of consultations. Datas for the administrative boundary were provided by Ministry of Territory Planning of Benin (https://gadm.org/download_country_v3.html).

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Table 2. Results of mobile consultations for skin NTDs in Plateau and Ouémé in Benin.

| Consultation period | Period 1 (Nov 6 to Dec 20, 2018) No. of cases—(%)—detection rate for 10,000 inhabitants | Period 2 (May 2 to Aug 17, 2019) No. of cases—(%)—detection rate for 10,000 inhabitants | Period 3 (Dec 1 to 21, 2020) No. of cases—(%)—detection rate for 10,000 inhabitants |
|---|--|--|--|
| Total number of cases of skin diseases | 607 (100) 29.72 | 2,244 (100) 98.58 | 2,216 (100) 121.83 |
| <i>Neglected tropical diseases</i> | 40 (6.6) 1.96 | 136 (6.1) 5.97 | 470 (21.2) 25.84 |
| Buruli ulcer | 3 (7.5) 0.14 | 28 (20.6) 1.23 | 6 (1.3) 0.33 |
| Leprosy | 6 (15.0) 0.29 | 5 (3.7) 0.22 | 11 (2.3) 0.6 |
| Scabies | 29 (72.5) 1.42 | 92 (67.6) 4.08 | 450 (95.7) 24.74 |
| Lymphatic filariasis | 2 (5.0) 0.098 | 10 (7.4) 0.44 | 3 (0.6) 0.16 |
| Mycetoma | 0 | 1 (0.7) 0.044 | 0 |
| Yaws | 0 | 0 | 0 |
| Cutaneous leishmaniasis | 0 | 0 | 0 |
| Post-Kala azar dermal leishmaniasis | 0 | 0 | 0 |
| Onchocerciasis | 0 | 0 | 0 |

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with a combination of rifampicin and clarithromycin according to WHO recommendations [26]. Category 2 and 3 cases were treated at the CDTLUB of Pobè, due to the size of the lesions and the need for a physiotherapist to prevent disability, a surgeon for lesion debridement and skin grafts, and nutritional and psychosocial care. Category 1 cases were managed at decentralized treatment sites run by the CDTLUB in the community, in collaboration with nurses from district health centers. The management of patients with Buruli ulcer in the community was managed by a multidisciplinary team until complete healing was achieved.

Six cases of leprosy (15.0% of the skin NTDs) were diagnosed during the first period of mobile consultations (Fig 4B). In 2019, there were five cases of leprosy (3.6% of the skin NTDs). Finally, in 2020 11 cases were detected (2.3% of the skin NTDs). Characteristics of patients presenting leprosy are presented in Table 3. Leprosy cases were treated according to WHO recommendations [27]. Multidrug treatment regimens consisting of combinations of rifampicin, clofazimine and dapsone were used, for six months in paucibacillary cases, and for 12 months in multibacillary cases. Treatment was initiated at the CDTLUB of Pobè and continued on an outpatient basis. Patients were made aware of the importance of treatment for preventing leprosy-related disabilities and the need to complete treatment. They also received psychosocial support.

In addition, 15 cases of lymphatic filariasis, were diagnosed, two during the first period of mobile consultations (5.0% of skin NTDs), 10 cases (7.2%) during the second period of mobile consultations and three (0.6%), during the third period of mobile consultations. All cases were diagnosed during a phase in which complications had already occurred and they were managed by the CDTLUB of Pobè. They were systematically treated with a combination of albendazole and ivermectin [28] and were advised on how to care for their limbs and prevent complications, such as chronic ulcers, leg erysipelas, and secondary bacterial infections.

One patient (0.7%) was diagnosed with mycetoma, during the second period of mobile consultations (Fig 4C). He was treated with cotrimoxazole for one year.

Finally, all 571 patients diagnosed with scabies during the three periods of mobile consultations (Fig 4D) received free local antiseptics for total body cleansing twice daily (in the morning and evening), antihistamines to reduce the associated pruritus, ivermectin (dose according to weight) and a solution of benzyl benzoate for topical application.

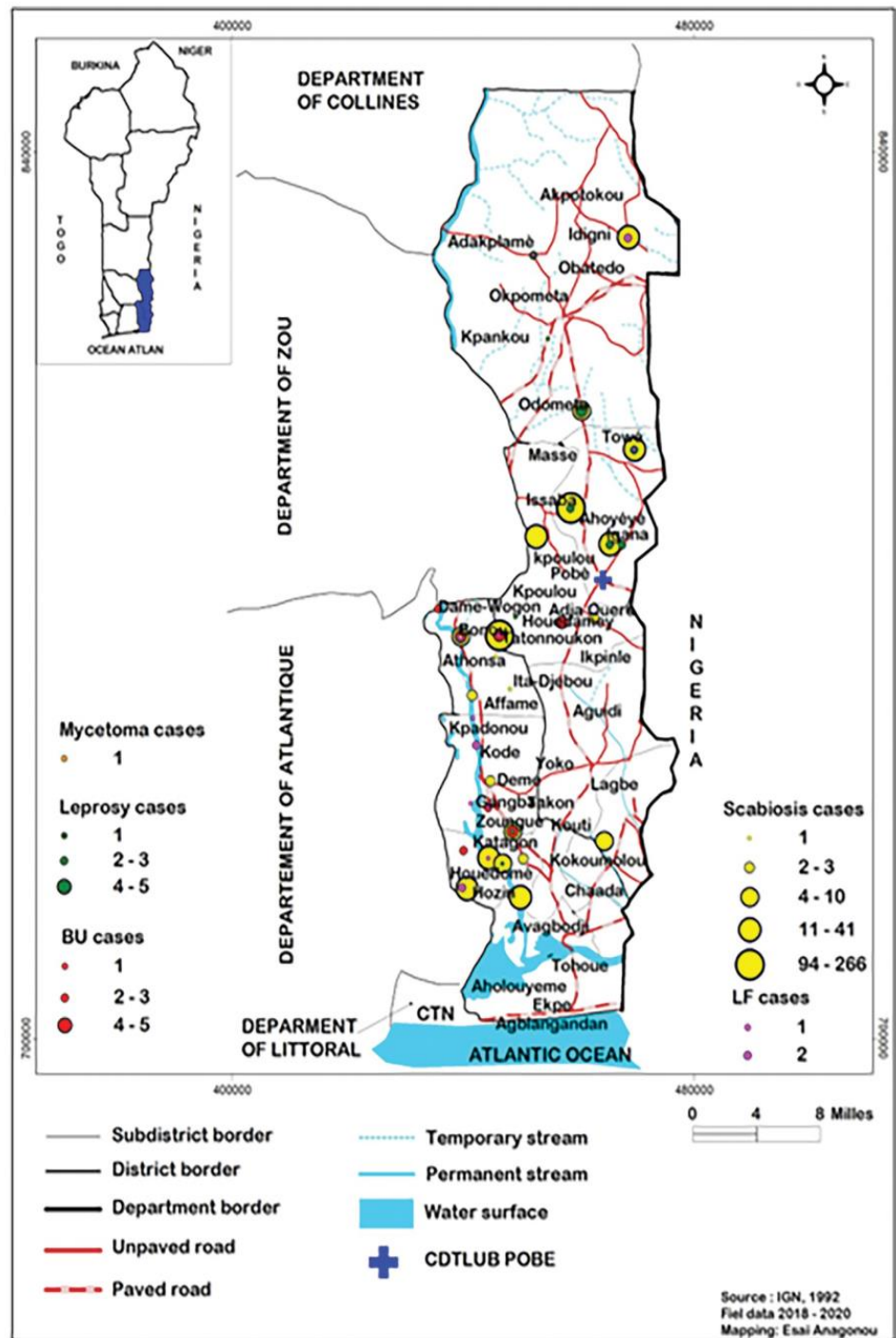


Fig 3. Localization of sites of consultations with skin NTDs. In green, leprosy cases, in red, Buruli ulcer (BU) cases, in yellow, scabies cases, in pink, lymphatic filariasis (LF) cases, in orange, mycetoma cases. Datas for the administrative boundary were provided by Ministry of Territory Planning of Benin (https://gadm.org/download_country_v3.html).

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3.3 Diagnosis of other skin diseases

Most of the lesions diagnosed during the three periods of mobile consultations were of infectious origin: 67.9% in 2018, 72.4% in 2019 and 78.6% in 2020. Infections were mycotic in 46.6%, 52.9% and 52.6% of cases, respectively, in 2018, 2019 and 2020, respectively. A summary of all the skin diseases encountered is presented in **Table 4**. Ringworm and epidermophytia were the two major non-NTD skin diseases diagnosed, accounting for 18.6% and 12.7%

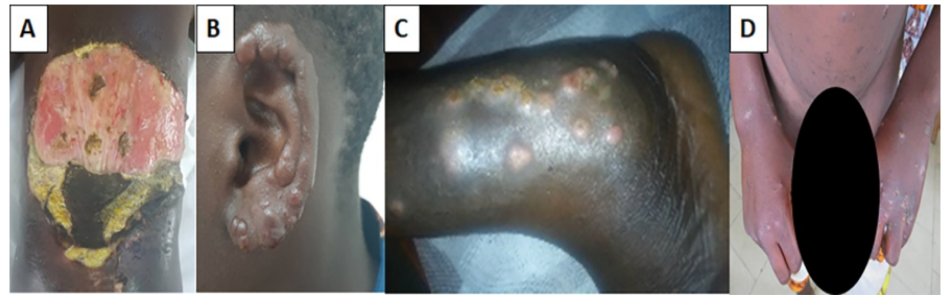


Fig 4. Neglected tropical skin diseases seen during mobile consultations. A) Buruli ulcer, B) leprosy, C) mycetoma, D) scabies. **Commentary about the illustrations and figures.** All the illustrations and figures are our own. They were taken during the implementation phase of this approach to integrate the fight against neglected tropical diseases with skin manifestations in the departments of Ouémé and Plateau in Benin from 2018 to 2020. We had taken them after obtaining informed consent and verbal authorization from all those who were considered. They were also informed and gave a verbal approval for the use of these iconographies in the framework of the valorization of this manuscript.

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of all consultations in the first period, 18.4% and 23.7%, respectively, in the second period and 28.7% and 13.9%, respectively, in the third period. Other frequent non-NTD skin diseases included impetigo contagiosa, pityriasis versicolor, chronic ulcers and eczema. Most of the non-infectious skin diseases were immunoallergic conditions (**Table 4**).

Depending on the type of skin dermatosis identified, its severity and, above all, the availability of an adequate technical platform, patients received outpatient medical treatment or were referred to a specialist structure.

IV Discussion

Skin NTDs are among the most prevalent infectious diseases in tropical and subtropical regions. They are difficult to manage due to the similarity of their clinical signs, their frequent co-endemicity and their occurrence in people living in poor regions with little or no access to healthcare. Integrated approaches are required to reduce the transmission of these diseases and to ensure that patients are managed earlier. In this context, we decided to develop a system of mobile consultations in two *départements* of Benin in which Buruli ulcer and leprosy are known to be highly endemic.

Our results indicate that these mobile consultations were highly successful. Indeed, in addition to the diagnosis and treatment of 646 cases of skin NTDs, the medical staff examined more than 5,200 people carrying various skin affections always with the aim of no exclusion or discrimination. The consultations were realised in respect of the gender approach, of the human person and of his dignity. Management of other skin diseases was also performed as optimally as possible and, if needed, the patients were referred to specialists. Furthermore, these consultations allowed us to see former patients treated at CDTLUB, pobè, allowing us to verify their scars and reinforcing our presence with these populations.

The mobile consultations provided useful epidemiological information about skin NTDs and other skin diseases in Plateau and Ouémé. This study provided us with a precise idea of the distributions of the skin diseases encountered, by administrative area and the sociodemographic and clinical characteristics of patients. Our study also provided a general overview of the most common skin NTDs and their stage of progression in the population. Similar experiments in the implementation of integrated management for skin NTDs have been performed in other countries, including Côte d'Ivoire, where Koffi et al. screened 2,310 individuals with skin lesions and diagnosed Buruli ulcer, leprosy and yaws in three health districts co-endemic for Buruli ulcer and leprosy [29].

Table 3. Characteristics of patients diagnosed with Buruli ulcer and leprosy during the three periods of mobile consultations held in the Plateau and Ouémé in Benin.

| | Buruli ulcer | Leprosy |
|---|---|--|
| Total number of cases | 37 | 22 |
| ≤ 15 years / > 15 years no. (%) | 14 (37.8) / 23 (62.2) | 4 (18.2) / 18 (81.8) |
| Sex ratio M/F [no. (%)] | 0.762 [16/21 (43.2/56.8)] | 1.2 [12/10 (54.5/45.5)] |
| Profession no. (%) | | |
| Learner | 13 (35.1) | 2 (9.1) |
| Merchant | 5 (13.5) | 2 (9.1) |
| Farmer | 8 (21.6) | 13 (59.1) |
| No profession | 1 (2.7) | - |
| Artisan | 5 (13.5) | 2 (9.1) |
| Household | 5 (13.5) | 3 (13.6) |
| Ethnic group no. (%) | | |
| Wémé and related | 26 (70.3) | 3 (13.6) |
| Holly | 8 (21.6) | 14 (63.6) |
| Nagot and related | 1 (2.7) | 5 (22.8) |
| Fon and related | 2 (5.4) | - |
| Educational level no. (%) | | |
| Educated | 13 (35.1) | 2 (9.1) |
| Not educated | 24 (64.9) | 20 (90.9) |
| Socio-economic level no. (%) | | |
| Medium | 1 (2.7) | 6 (27.3) |
| Low | 36 (97.3) | 16 (72.7) |
| Municipality no. (%) | | |
| Pobè | 4 (10.8) | 9 (40.9) |
| Kétou | - | 9 (40.9) |
| Dangbo | 10 (27.0) | 2 (9.1) |
| Adjohoun | 7 (18.9) | 1 (4.6) |
| Adja-Ouèrè | 8 (21.6) | 1 (4.6) |
| Bonou | 7 (18.9) | - |
| Akpro-Missérété | 1 (2.7) | - |
| Bacilloscopy no. (%) | | |
| Positive | 11 (29.7) | 13 (59.1) |
| Negative | 26 (70.3) | 9 (40.9) |
| Buruli ulcer lesion type no. (%) | Ulceration 23 (62.2) Plaque 6 (16.2) Nodule 4 (10.8) Oedema 4 (10.8) | |
| Buruli ulcer WHO classification no. (%) | | |
| Category I | 17 (46.0) | |
| Category II | 11 (29.7) | |
| Category III | 9 (24.3) | |
| Leprosy clinical classification no. (%) | | |
| Grade 2 infirmity | | 12 (54.6) |
| Distribution | | ≤ 15 years 1 (4.5) / Women 5 (22.7) / Men 6 (27.3) |
| Leprosy microbiological classification no. (%) | | |
| Multibacillary | | 16 (72.7) |
| Paucibacillary | | 6 (27.3) |

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Our study highlighted several advantages of this approach. First, we showed that mobile consultations facilitated the on-site treatment of patients living in remote villages. This strategy helped to reduce the burden of diseases that remained widespread. Second, this study led to the detection of a large number of skin diseases, including neglected diseases that had not been declared to health centers and were particularly widespread in remote areas. It therefore

Table 4. Skin diseases observed during mobile consultations in Plateau and Ouémé in Benin.

| Skin disease | Period 1 (Nov 6 to Dec 20, 2018) No. of cases (%) Total no. 607 | Period 2 (May 2 to Aug 17, 2019) No. of cases (%) Total no. 2,444 | Period 3 (Dec 1 to 21, 2020) No. of cases (%) Total no. 2,216 |
|-------------------------------------|--|--|--|
| Infectious skin diseases | 412 (67.9) | 1,769 (72.4) | 1,742 (78.6) |
| <i>Mycotic dermatosis</i> | 283 (46.6) | 1,293 (52.9) | 1,166 (52.6) |
| Ringworm scalp | 113 (18.6) | 449 (18.4) | 634 (28.6) |
| Epidermophytia | 77 (12.7) | 580 (23.7) | 308 (13.9) |
| Pityriasis versicolor | 55 (9.1) | 124 (5.1) | 122 (5.5) |
| Intertrigo | 16 (2.6) | 71 (2.9) | 42 (1.9) |
| Vulvovaginitis | 10 (1.7) | 21 (0.9) | 38 (1.7) |
| Onychomycosis | 8 (1.3) | 44 (1.8) | 20 (0.9) |
| Seborrheic dermatitis | 2 (0.3) | - | 2 (0.1) |
| Nappy rash | 2 (0.3) | 4 (0.1) | - |
| <i>Bacterial dermatoses</i> | 96 (15.8) | 342 (14.0) | 110 (5.0) |
| Impetigo contagiosa | 65 (10.7) | 187 (7.7) | 74 (3.3) |
| Leprosy | 6 (1.0) | 5 (0.2) | 11 (0.5) |
| Buruli ulcer | 3 (0.5) | 28 (1.2) | 6 (0.3) |
| Yaws | - | 2 (0.1) | - |
| Mycetoma | - | 1 (0.0) | - |
| Furuncle | 11 (1.8) | 71 (2.9) | 8 (0.4) |
| Osteomyelitis | 11 (1.8) | 22 (0.9) | 9 (0.4) |
| Erysipelas | - | 26 (1.0) | 1 (0.1) |
| Gonococcal urethritis | - | - | 1 (0.1) |
| <i>Parasitic dermatoses</i> | 31 (5.1) | 104 (4.3) | 454 (20.5) |
| Scabies | 29 (4.8) | 93 (3.8) | 450 (20.3) |
| Lymphatic filariasis | 2 (0.3) | 10 (0.4) | 3 (0.1) |
| Larva migrans | - | 1 (0.1) | 1 (0.1) |
| <i>Viral dermatoses</i> | 2 (0.3) | 30 (1.2) | 12 (0.6) |
| Herpes simplex | 2 (0.3) | 11 (0.5) | 2 (0.1) |
| Herpes zoster | - | 13 (0.5) | 8 (0.3) |
| Wart | - | 4 (0.2) | 1 (0.1) |
| Condyloma | - | 2 (0.0) | 1 (0.1) |
| Non-infectious skin diseases | 195 (32.1) | 675 (27.6) | 474 (21.4) |
| <i>Immunoallergic dermatoses</i> | 89 (14.7) | 287 (11.7) | 287 (13.0) |
| Prurigo | 39 (6.4) | 53 (2.2) | 119 (5.4) |
| Eczema | 23 (3.8) | 108 (4.4) | 88 (4.0) |
| Atopic dermatitis | 15 (2.5) | 87 (3.6) | 43 (1.9) |
| Palmoplantar keratoderma | 7 (1.2) | 23 (0.9) | 8 (0.4) |
| Urticaria | 4 (0.6) | 13 (0.5) | 24 (1.1) |
| Keratosis pilaris | 1 (0.2) | 3 (0.1) | 1 (0.1) |
| Fixed pigmented erythema | - | - | 4 (0.2) |
| <i>Tumoral dermatoses</i> | 38 (6.3) | 93 (3.8) | 44 (2.0) |
| Fibrosing folliculitis | 19 (3.1) | 19 (0.8) | 20 (0.9) |
| Keloid | 5 (0.8) | 46 (1.9) | 11 (0.5) |
| Lipoma | 5 (0.8) | 16 (0.6) | 6 (0.3) |
| Synovial cyst | 1 (0.2) | 7 (0.3) | 5 (0.2) |
| Hamartoma | 3 (0.5) | 1 (0.0) | 2 (0.1) |
| Metastatic tumor | 4 (0.7) | 4 (0.2) | - |

(Continued)

Table 4. (Continued)

| Skin disease | Period 1 (Nov 6 to Dec 20, 2018) No. of cases (%) Total no. 607 | Period 2 (May 2 to Aug 17, 2019) No. of cases (%) Total no. 2,444 | Period 3 (Dec 1 to 21, 2020) No. of cases (%) Total no. 2,216 |
|--------------------------------|--|--|--|
| Syringoma | 1 (0.2) | - | - |
| <i>Inflammatory dermatoses</i> | 32 (5.3) | 60 (2.5) | 57 (2.6) |
| Acne | 14 (2.3) | 12 (0.5) | 18 (0.8) |
| Sudamina (miliaria) | 11 (1.8) | 36 (1.5) | 17 (0.8) |
| Lichen | 4 (0.7) | 8 (0.3) | 15 (0.7) |
| Alopecia areata | 2 (0.3) | 0 (0) | 3 (0.1) |
| Psoriasis | 1 (0.2) | 4 (0.2) | 4 (0.2) |
| <i>Ulcerative dermatoses</i> | 27 (4.4) | 212 (8.7) | 71 (3.2) |
| Chronic non-BU ulcer | 24 (3.9) | 212 (8.7) | 65 (2.9) |
| Plantar perforating pain | 3 (0.5) | - | 6 (0.3) |
| <i>Genodermatoses</i> | 6 (1.0) | 9 (0.4) | 4 (0.2) |
| Neurofibromatosis | 3 (0.5) | 8 (0.3) | 3 (0.1) |
| Congenital ichthyosis | 2 (0.3) | 1 (0.1) | 1 (0.1) |
| Ainhum disease | 1 (0.2) | - | - |
| <i>Autoimmune dermatoses</i> | 2 (0.3) | 11 (0.5) | 3 (0.1) |
| Vitiligo | 2 (0.3) | 10 (0.4) | 2 (0.1) |
| Lupus erythematosus | - | 1 (0.1) | 1 (0) |
| <i>Vascular dermatoses</i> | 1 (0.2) | 3 (0.1) | 2 (0.1) |
| Tuberous hemangioma | 1 (0.2) | 3 (0.1) | 2 (0.1) |
| <i>Other dermatoses</i> | - | - | 6 (0.3) |
| Pellagra | - | - | 2 (0.1) |
| Scrotal calcinosis | - | - | 2 (0.1) |
| Exogenous ochronosis | - | - | 2 (0.1) |

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provided new data that could be used to raise awareness among associations and government institutions, because the data obtained in hospitals and health centers only partially reflects the reality on the ground and does not accurately represent the true burden of these diseases. Third, we observed an increase in the number of people agreeing to take part in mobile consultations between periods 1 and 3. This may be due to an increase in awareness about these diseases and the availability of free treatment for patients suffering from them. A relationship of trust was also established between the population and the medical team over the three periods of consultation.

Mobile consultations have brought a change of paradigm in the care of patients. Indeed, usually, it was the patient who went to the doctor but through this approach, it was the caregivers who went to the patients. Thus, health care was made with quality, was accessible to these populations in remote areas and participated in reducing the burden of morbidity linked to NTDs in these localities. This approach also was developed in a more human approach since taking into account the low standard of living of the population, consultation were made free and the management of cases was ensured. Finally, human, material, financial and time resources were mutualized and rationalized to screen for several skin NTDs and many other skin diseases in order to be as most efficient as possible.

Buruli ulcer diagnosis by PCR was introduced in Benin in 2004 following an increase in the number of cases in the country [30,31]. This infectious disease is characterized by painless skin lesions that can lead to scarring, contracture deformities, amputations and disabilities if left

untreated [31]. Along with Côte d'Ivoire, Ghana and Cameroon, Benin is one of the four African countries in which Buruli ulcer is most endemic. Between 2006 and 2015, the CDTLUB of Pobè diagnosed and treated 1,365 patients, 69% of whom were from Ouémé, with the other 31% from Plateau [32]. During the mobile consultations in this study, 37 patients were diagnosed and treated. Most of the cases of Buruli ulcer detected during the three consultation periods were from Ouémé, which is crossed by the River Ouémé. There is an alternation of dry and wet seasons in this *département*. During wet seasons, a large area around the river is flooded, resulting in large expanses of stagnant water, which constitute the main ecological niche of the bacillus, creating an environment in which the disease is predominant [33].

Scabies remained the leading skin NTD, accounting for the highest proportion of cases identified, over these three years of mobile consultations. This disease constitutes a real public health problem because of its high prevalence, and its high morbidity in children [34]. For the 5,267 people presenting skin diseases during the three years of mobile consultations, the overall prevalence of scabies was 10.8%, with most cases occurring in children under the age of 15 years. In 2020, the consultation team came across community cases of scabies, initially in the Pobè municipal area and, more specifically, in the district of Issaba, and subsequently in the Adja-Ouèrè municipal area, in which the incidence of scabies was very high. Almost everyone, including the healthcare agents, had scabies in these areas. This observation led us to alert the local health authorities and the Ministry of Health, which organized mass treatment with ivermectin and made benzyl benzoate solution available to healthcare facilities to ensure correct case management.

Associations of scabies, impetigo and superficial mycoses are often attributed to developing countries, a lack of hygiene being the factor common to these three infections in the vulnerable populations of these countries [35,36]. We also found the prevalence of these three infections to be high. A specific strategy should therefore be implemented to target dermatoses of this type by raising the awareness of the population concerning the importance of maintaining good physical hygiene.

We also detected 22 cases of leprosy during the three years of mobile consultations, this disease being the third most frequent after scabies and Buruli ulcer. Leprosy is a chronic disease caused by a slow-growing bacterium, *Mycobacterium leprae*. The consultations in 2018 and 2020 detected the largest numbers of leprosy cases (6 cases in 2018 and 11 cases in 2020), corresponding to a prevalence of 0.08 and 0.11%, respectively. These values indicate that Benin remains among the countries considered to have eliminated leprosy, as the prevalence of this disease remains below 1 per 10,000 inhabitants. Nevertheless, this disease remains a major health challenge due to the large proportion of multibacillary cases and the increase in the number of individuals newly registered with grade 2 disabilities. In this context, it is important to strengthen the epidemiological surveillance of leprosy and to encourage its early detection in populations at risk, by adopting new strict strategies, such as the implementation of diagnostic tools for detection of the bacillus and for the detection of resistance genes.

The declaration of skin NTDs to health centers is not compulsory, accounting for the often poor knowledge of healthcare workers concerning these diseases [37]. In addition to the large distances between health centers and villages, the poor quality of roads, the lack of transport vehicles, and the high rates of very severe poverty in these populations [38], the painless nature of certain types of skin NTDs, such as Buruli ulcer, mycetoma, cutaneous leishmaniasis, yaws and some forms of leprosy, is an important factor underlying their neglected nature [39–43]. If untreated, these diseases progress to severe forms, leading to permanent physical disability altering quality of life (work, education, mental state, etc.), highlighting the importance of mobile consultations for managing patients.

The consultation team encountered several problems that lowered its efficiency. For example, it was difficult to pick up the patients affected by diseases in very remote regions, particularly as the consultations took place during the rainy season, when it was difficult to cross rivers without motorized boats. Moreover, shortages of medical consumables for basic necessities, such as antifungal drugs (oral and topical), antiparasitic agents and topical corticosteroids for patient care were also identified as problematic and a source of concern for the team. Finally, some local actors were found to be less implicated in the project and a lack of means for raising awareness was also noted.

Based on the results obtained, those involved in the epidemiological surveillance of skin NTDs are faced with a number of challenges. Action will be required to meet these challenges without jeopardizing the chances of achieving the objectives of the new roadmap developed by the WHO for 2030 [44]. It will be important:

- i. To ensure the maintenance of the achievements attained by implementing this approach in Ouémé and Plateau through social mobilization, training of health workers on screening and integrated management of NTDs, wound care and encouraging the routinization of this approach to take into account a large number of people,
- ii. To lobby political and administrative authorities at various levels to get them to increase the budget allocated to the fight against tropical skin diseases. Efforts should be directed towards encouraging the construction of sociosanitary infrastructures in these isolated rural areas to facilitate the access of these populations to primary healthcare and to meeting the objectives of sustainable development (ensuring universal access to medical coverage and health services). Conversely, it will also be important to integrate activities for combating skin NTDs into the primary healthcare activities offered to populations by peripheral healthcare centers. For example, when performing vaccinations or routine prenatal consultations, healthcare workers should systematically look for a change in the color or relief of the skin,
- iii. To intensify activities for raising awareness, to change the behavior of the population in terms of risky behaviors contributing to the occurrence of these diseases,
- iv. To implement activities designed to promote health,
- v. To expand the scope of implementation of mobile clinics to other regions of Benin in which these diseases are endemic. If resources permit, non-endemic areas that have remained “silent” epidemiologically should also be considered.

Another crucial transverse component must also be added to all these activities to fight disease or reduce the burden of skin NTDs: water, hygiene and sanitation, as most of these diseases are favored by a lack of hygiene and environments conducive to their occurrence. This is why one of the strategic challenges in the fight against NTDs in Ouémé and Plateau will be making clean drinking water available in remote areas. We also need to improve the access of the population to healthcare facilities, to construct latrines, and to destroy the breeding grounds of disease. In other words, we need to clean up the environment to make it healthy, viable and livable, to guarantee the health of the population. The intervention and involvement of other health-related sectors will be essential to achieve this goal. The challenge will therefore be to federate the actions of the Ministry of Health, the Ministry of the Environment and the Living Environment, the Ministry of Water and Electricity, the Ministry of Education (to improve the level of education of the population and to promote the enrolment of all school-age children), the Ministry of Agriculture and Fisheries and the Ministry of Finance (which could promote small income-generating activities to lift the population out of poverty). All of

these sectors must act holistically on the other determinants of health. In other words, the challenge will be to combine the integrated fight against these diseases with the implementation of the "One Health" concept.

V. Conclusion

Mobile consultations are a highly efficient strategy for the integrated screening of skin diseases, particularly NTDs with skin manifestations, which are often diagnosed late. Sustaining these activities could help to reduce the burden of NTDs and contribute to the achievement of the objectives of the new WHO roadmap developed for the 2030 horizon.

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PARTIE 3 :

**Mise en place du kit GenoType LepraeDR à
Pobè au Bénin, pour la détection de la
résistance aux antituberculeux chez
*Mycobacterium leprae***

3.1. Introduction

La lèpre est une maladie infectieuse chronique causée par *Mycobacterium leprae*, une mycobactérie. Elle se manifeste par des lésions de la peau, des nerfs périphériques, de la muqueuse des voies respiratoires supérieures et des yeux. En l'absence de traitement, cette maladie peut être très mutilante. La bactérie a été découverte en 1873 par Armauer Hansen à Bergen, raison pour laquelle la lèpre est également appelée maladie de Hansen (28).

En 1991, l'OMS avait défini comme objectif l'élimination de la lèpre en tant que problème de santé publique au niveau mondial, cette élimination étant définie comme une prévalence inférieure à un cas pour 10,000 habitants. Cet objectif a été atteint en 2000. Cependant, les chiffres sont sous-estimés, étant donné qu'il s'agit d'une maladie «tabou» dans de nombreux pays. Aussi, la lèpre demeure un problème de santé publique selon les données provenant de 103 pays du monde (29).

La lèpre peut conduire à des formes d'incapacités sévères aboutissant à une stigmatisation sociale. Aussi, est-il important de mettre en place un dépistage et un traitement précoce. Le diagnostic est avant tout clinique, mais une confirmation microbiologique est toutefois possible. Concernant le traitement, l'OMS recommande une polychimiothérapie afin de lutter contre la résistance bactérienne (29). En 1964 a été décrite la première résistance (30). Au cours du temps, d'autres résistances aux molécules de première puis de deuxième ligne sont apparues, et des cas de multi-résistance ont été décrits (31). Afin d'endiguer ce problème de santé publique, l'OMS a mis en place un réseau sentinelle de surveillance des résistances dans plusieurs pays endémiques ainsi qu'un guide pour la surveillance de la résistance aux antilépreux (32).

Étant donné l'impossibilité de cultiver *M. leprae* sur les milieux de culture, la recherche de la résistance aux anti-lépreux se fait principalement à l'aide d'outils de biologie moléculaire. Les principales résistances sont identifiées au niveau de trois gènes, *rpoB*, *gyrA* et *folP1* qui codent respectivement pour les cibles de la rifampicine, la dapsonne et les fluoroquinolones. Ces molécules constituent le traitement de première intention utilisées dans la polychimiothérapie (33). Ces mutations peuvent être détectées par séquençage Sanger, cependant il est très difficile d'installer cette technique dans les pays endémiques. Le kit GenoType LeptraeDR (Hain Lifescience) est un kit développé en 2012 permettant de détecter les mutations les plus fréquentes dans ces 3 gènes. Ce test, aisé d'utilisation, est disponible sur le marché. Il peut être installé dans les pays où l'accès au séquençage des gènes de résistance n'est pas possible (34).

Le Bénin est un pays d'Afrique de l'Ouest considéré comme endémique pour la lèpre (35). Selon l'OMS, 77 cas de lèpre ont été enregistrés dans le pays en 2021 (36). Entre 2009 et 2015 le pays a signalé plusieurs cas de résistance aux antiléproux de première intention (37). La mise en place du test GenoType LepaeDR permettant de détecter ces résistances, peut être un énorme atout pour une prise en charge optimale des patients.

En 2012, notre équipe de recherche ATOMycA a installé un laboratoire de biologie moléculaire dans un centre de diagnostic et de traitement de la lèpre et de l'ulcère de Buruli (CDTLUB) de Pobè, au sud du Bénin. En 2016 et grâce à l'expertise acquise par les techniciens du laboratoire de Pobè, la PCR en temps réel ciblant la séquence RLEP, spécifique à *M. leprae*, a été développée (38). Cependant le diagnostic de la résistance aux antiléproux n'était pas proposé aux médecins.

Dans ce contexte, les objectifs de ce projet étaient : i) de tester le kit GenoType LepaeDR au laboratoire de bactériologie du CHU d'Angers afin de pouvoir répondre à la problématique de la résistance aux anti-léproux des patients diagnostiqués à *M. leprae* au CDTLUB de Pobè, ii) de rédiger une procédure complète permettant au laboratoire du CDTLUB de Pobè de mettre en place ce test et de pouvoir en interpréter les résultats.

3.2. Diagnostic de la lèpre

3.2.1. Diagnostic clinique

Le diagnostic de la lèpre est avant tout clinique. Le patient présente une ou plusieurs tâches pouvant évoluer sur tout le corps, d'aspect pâle, rougeâtre ou de couleur cuivrée. Ces tâches peuvent être plates ou en relief, elles ne démangent pas. Une perte nette de sensibilité à la chaleur, au toucher ou à la douleur est observée.

La réponse immunitaire du sujet atteint détermine la forme clinique de la lèpre. Ainsi, on distingue la forme tuberculoïde, appelée également Pauci-Bacillaire (PB) et la forme lépromateuse, dite Multi-Bacillaire (MB). La forme PB résulte d'un profil Th1 avec une immunité cellulaire adaptée et une réaction inflammatoire localisée. Peu ou pas de bacilles sont observés en microscope (0 à 10 bacilles/ 100 champs microscopiques) et les cytokines retrouvées sont l'interleukine-2 et 12 et l'interféron gamma. La forme PB est la plus fréquente,

elle est non contagieuse et se caractérise par la présence de moins de 5 lésions, sous formes des tâches de dépigmentation.

La forme MB quant à elle résulte d'un profil Th2 avec une mauvaise réponse immunitaire et une réaction inflammatoire disséminée, accompagnée d'un fort titre d'anticorps. De nombreux bacilles sont observés (1 à 10 bacilles/ 10 champs jusqu'au > 1000 bacilles/ champ microscopique) et les cytokines retrouvées sont l'interleukine- 4,5,6,10 et 13. La forme MB est moins fréquemment retrouvée mais elle est contagieuse. Elle se caractérise par la présence de plus de 5 lésions d'aspect papules ou pustules luisantes sur tout le corps (39,40,41). Le **Tableau 1** résume les caractéristiques des deux formes cliniques de la lèpre.

Tableau 1 Résumé des caractéristiques de 2 formes cliniques de lèpre.

| Caractéristiques | Lèpre tuberculoïde ou Pauci-Bacillaire (PB) | Lèpre lépromateuse ou Multi-Bacillaire (MB) |
|---------------------------------|--|--|
| Nombre de lésions | <5 lésions | >5 lésions |
| Fréquence | +++ | + |
| Caractéristique clinique | Taches de dépigmentation | Papules ou pustules luisantes sur tout le corps |
| Profil Th | Th1 | Th2 |
| Réponse immunitaire | Cellulaire | Inadéquate avec fort titre d'anticorps |
| Réaction inflammatoire | Localisée | Disséminée |
| Nombre de bacilles | Peu de bacilles | Prolifération massive des bacilles |
| Cytokines | IL-2, INF- γ , IL-12 | IL-4, IL-5, IL-6, IL-10, IL-13 |
| Caractère contagieux | Non contagieuse | Contagieuse |

3.2.2. Diagnostic microbiologique

Le bacille de la lèpre est préférentiellement retrouvé dans les zones les plus froides du corps, en particulier au niveau du lobe de l'oreille ou encore dans les narines. Ces deux sites anatomiques seront ainsi préférentiellement prélevés pour réaliser le diagnostic biologique. *M. leprae* est non cultivable *in vitro* et sa durée de doublement est extrêmement longue, estimée à environ 2 semaines (28,42).

Une confirmation microbiologique du diagnostic clinique est possible. En zone d'endémie, la coloration de Ziehl Neelsen est utilisée. Depuis quelques années a été développée la confirmation microbiologique par PCR. La principale cible est la séquence d'insertion RLEP, présente en 37 copies (43). Cette séquence est spécifique de *M. leprae* (44,45).

3.3. Traitement de la lèpre

Dans les années 1940, le premier antilépreux à avoir été mis sur le marché a été la dapsonne (46). Cette molécule inhibe la synthèse des folates en agissant sur la Dihydroptéroate Synthétase (DHPS) codée par le gène *folP*, et bloquant ainsi la synthèse d'acide folique, composant indispensable à la croissance de la bactérie (47). En 1964, les premières résistances à la dapsonne ont été décrites (30). Dans le but de limiter l'émergence de résistances liées à cette monothérapie l'OMS a mis en place polychimiothérapie en 1981. Elle associe, en premier ligne, la rifampicine (600 mg/mois) et la dapsonne (100 mg/jour) pour 6 mois en cas de lèpre PB, ou, la rifampicine (600 mg/mois), la dapsonne (100 mg/jour) et la clofazimine (300 mg/mois et 50 mg/jour) pour 12 mois en cas de lèpre MB, une thérapie qui a permis de diminuer l'incidence et la prévalence de la lèpre au niveau mondial (29). La rifampicine possède une activité bactéricide importante. Elle bloque la sous-unité bêta de l'ARN polymérase ADN dépendante codée par le gène *rpoB* (48). La clofazimine possède une activité bactériostatique, avec un mécanisme encore assez mal connu à ce jour.

Des molécules de seconde ligne sont utilisées en cas de résistance à celles de première ligne. Ainsi, en cas de résistance à la rifampicine seule ou de double résistance à la rifampicine et à la dapsonne, la thérapeutique anti-lépreuse doit associer la minocycline (100 mg/jour) avec

l'ofloxacine (400 mg/jour) et la clofazimine (50 mg/jour) pour 6 mois suivi d'une combinaison d'ofloxacine (400 mg/jour) ou de minocycline (100 mg/jour) avec la clofazimine (50 mg/jour) pour 18 mois. D'autres formes de résistance existent mais n'impliquent pas le changement de la polychimiothérapie standard (32). La **figure 1** résume les traitements recommandés dans les différentes situations.

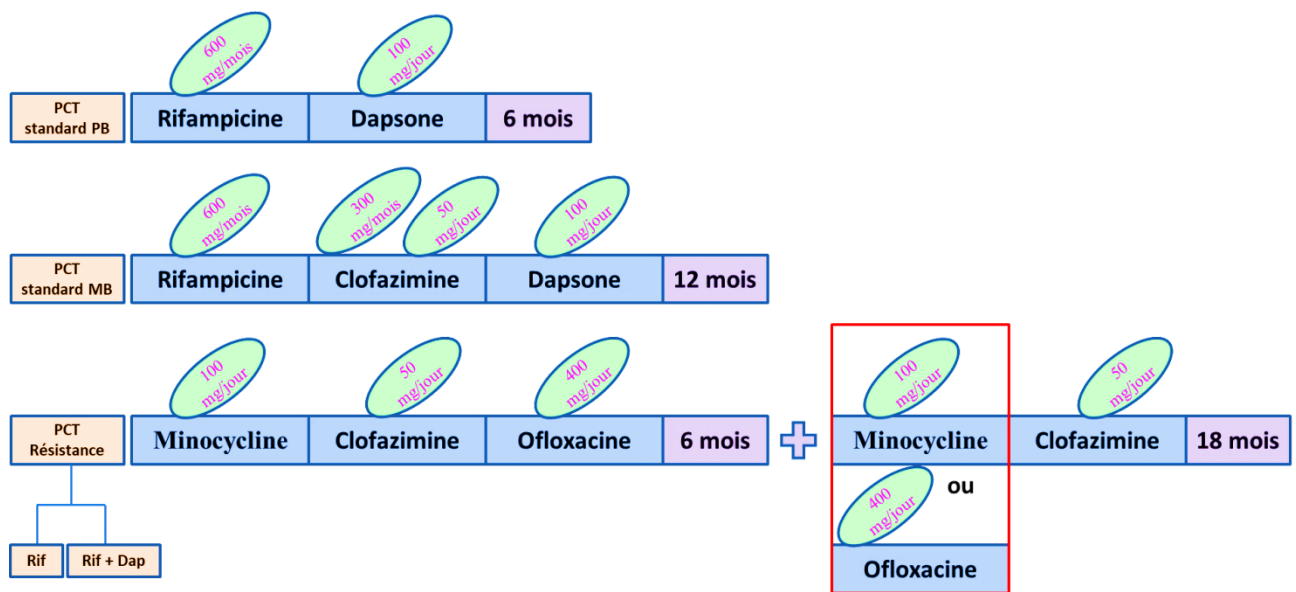


Figure 1 Présentation des différents traitements antilépreux recommandés, selon l'OMS.

3.3. Résistance aux antilépreux

La résistance à la dapsone a été notée dès 1964 (30), celle à la rifampicine dès 1976. Par la suite, des résistances aux quinolones ont aussi été mises en évidence (49). Plusieurs multi- résistances, définies comme la résistance combinée à plusieurs antilépreux, ont été décrites (31). A ce jour, il n'a pas encore été décrit de résistance à la clofazimine. Cependant, la cible de cette molécule n'est pas bien connue, rendant compliquée sa recherche.

Les principales mutations conférant la résistance aux antilépreux sont présentées dans le **tableau 2**.

Tableau 2. Principales mutations dans les gènes *rpoB*, *folP1* et *gyrA*, conférant des résistances à la rifampicine, à la dapsonne et à l’ofloxacine, chez *M.leprae* (33).

| Antilépreux | Protéine cible | Mutations | Fréquence |
|-------------|----------------|--|---------------|
| Rifampicine | <i>rpoB</i> | His451Tyr, Ser456Leu | Fréquente |
| | | Ser456Met, Ser456Phe | Peu fréquente |
| | | His431Asp , Gly432Ser, Gln438Val, Phe439 (Lys + Phe insert) /Met440, Asp441Asn, Asp441Tyr, Ser447Cys, His451Asp, His451Val, Ser456Trp, Leu458Val, Leu458Pro, Gly459Ser | Rare |
| Dapsonne | <i>folP1</i> | Thr53Ala, Thr53Ile, Pro55Arg, Pro55Leu | Fréquente |
| | | Thr53Arg, Thr53Val, Pro55Ala, Thr53Val, Pro55Ser | Rare |
| Ofloxacine | <i>gyrA</i> | Gly89Cys | Rare |
| | | Ala91Val | Fréquente |

3.4. Le kit GenoType Leprae DR pour la détection de la résistance aux antilépreux.

3.4.1. Principe du kit

Le GenoType LepraeDR (VER 1.0, Hain LifeScience) est un test qualitatif de diagnostic *in vitro* pour l’identification de *M. leprae* et la détermination de sa résistance à la rifampicine et/ou l’ofloxacine et/ou la dapsonne. Il possède un marquage CE IVD. Ce test peut être utilisé à partir d’échantillons cutanés positifs à la coloration de Ziehl Neelsen.

- La présence de *M. leprae* est déterminée par la détection de la séquence répétée RLEP.
- La résistance à la rifampicine est déterminée par la détection des principales mutations du gène *rpoB*, codant pour la sous-unité bêta de l’ARN polymérase.

- La résistance à l'ofloxacine ou aux autres fluoroquinolones est déterminée par la détection par la principale mutation du gène *gyrA*, codant pour la sous-unité A de l'ADN gyrase.
- La résistance à la dapsonne est déterminée par la détection de mutations dans le gène *folP1*, codant pour la dihydroptéroate synthétase.

Le test GenoType LepraeDR VER 1.0 est basé sur la technique DNA•STRIP et comporte trois phases (**figure 2**) :

Phase A : extraction de l'ADN à partir d'échantillons positifs à la coloration de Ziehl Neelsen

Phase B : amplification multiplex à l'aide d'amorces biotinylées

Phase C : hybridation inverse sur bandelette avec le TwinCubator

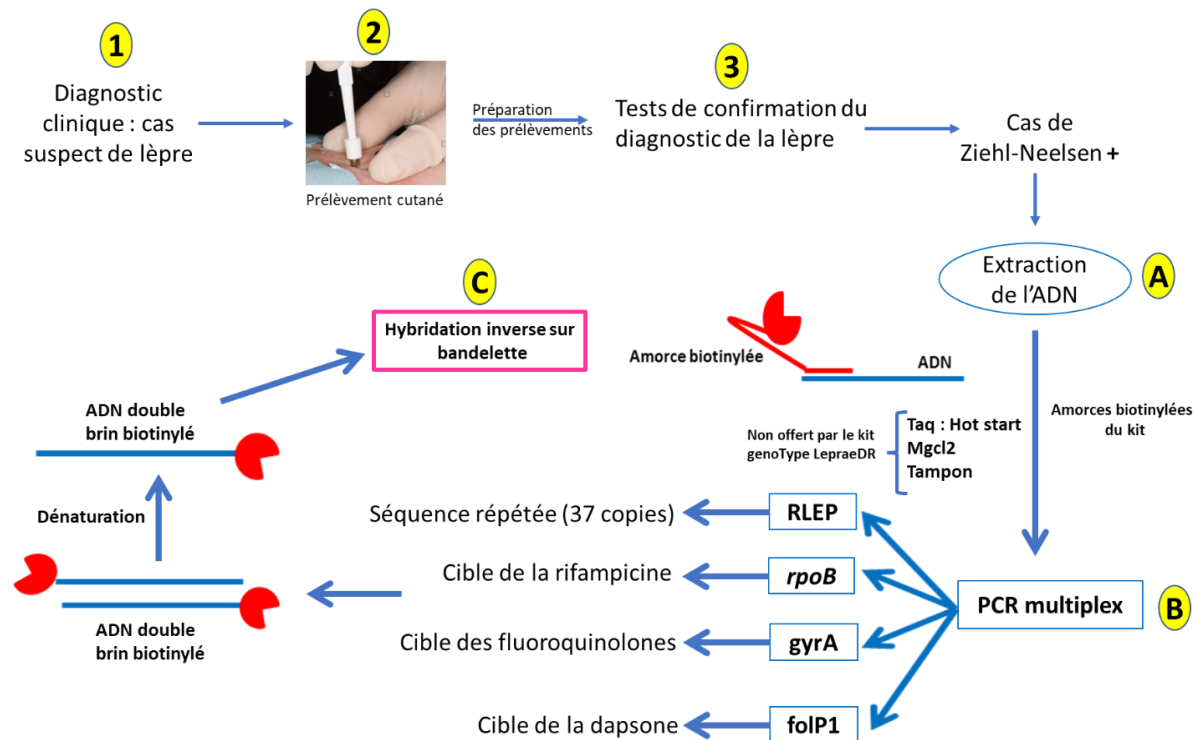


Figure 2. Stratégie d'utilisation du test GenoType LepraeDR

Le Mélange Amorces/Nucléotides, contenu dans le kit, contient des amorces biotinylées pour l'amplification de régions spécifiques du génome de *M. leprae* (RLEP, *rpoB*, *gyrA* et *folP1*).

Les bandelettes sont recouvertes de sondes spécifiques complémentaires aux acides nucléiques amplifiés. Après dénaturation chimique, les amplicons simple brin formés se lient aux sondes par hybridation. Sous l'action de conditions stringentes, les molécules d'ADN simple brin se lient spécifiquement à leur brin complémentaire, permettant ainsi aux sondes de différencier plusieurs mutations de séquences dans les régions des gènes examinées. Le conjugué streptavidine-phosphatase alcaline est ensuite immobilisé sur la membrane par l'intermédiaire de la biotine présente sur chaque amplicon formé. Enfin, la phosphatase alcaline transforme un substrat ajouté en colorant qui forme un précipité coloré sur la bandelette. Les signaux obtenus sont facilement et rapidement interprétés à l'aide d'une matrice fournie avec chaque kit.

3.4.2. Interprétation des résultats

Chaque bandelette contient 16 zones réactionnelles ainsi qu'un marquage coloré permettant son orientation (**figure 3**).

Six bandes permettent de contrôler le bon déroulement du test :

- une bande *Contrôle Conjugué* (CC) qui permet de contrôler la fixation du conjugué sur la bandelette et le bon déroulement de la révélation chromogénique.
- une bande *Contrôle Universel* (UC) qui détecte les espèces connues de mycobactéries ainsi que les bactéries Gram-positives riches en G+C.
- une bande ML pour *Mycobacterium leprae* qui permet de confirmer la présence de *M. leprae* dans le prélèvement.
- une bande de contrôle du locus *rpoB*, une de contrôle du locus *gyrA* et une de contrôle du locus *folP1*.

La présence de ces 6 bandes contrôles (CC, UC, ML, contrôle *rpoB*, contrôle *gyrA* et contrôle *folP1*) est obligatoire pour analyser les résultats. L'intensité de la bande ML varie selon la concentration d'ADN de départ, si cette bande n'est pas positive, l'échantillon ne peut pas être interprété.

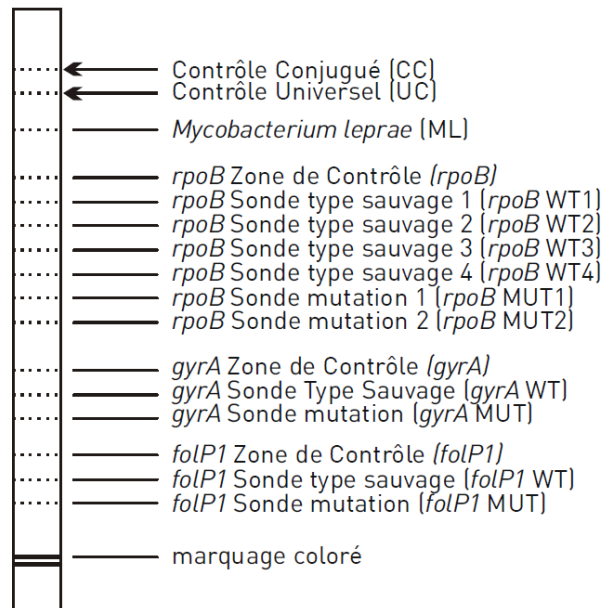


Figure 3. Représentation d’une bandelette du kit GenoType LepraeDR avec l’emplacement des sondes.

Les sondes type sauvage (WT) comprennent les séquences non mutées de chaque région d’intérêt du gène testé. En présence d’une mutation, l’amplicon produit ne peut pas s’hybrider à la sonde de type sauvage correspondante. Ainsi, l’absence de signal dans au moins une des sondes type sauvage indique la présence d’une résistance à l’antituberculeux correspondant. Lorsque tous les types sauvages d’un même gène sont positifs, il n’y a pas de mutation détectable au sein des régions analysées. La souche testée est alors considérée sensible pour l’antituberculeux correspondant.

Les sondes type mutant (MUT) comprennent les séquences qui correspondent aux mutations les plus fréquentes des 3 gènes à l’origine d’une résistance (**Tableau 3**).

Seules les bandes d’intensité environ égale ou supérieure à celle du Contrôle Universel (UC) doivent être prises en compte pour l’interprétation. Il est à noter une possibilité que l’échantillon testé contienne plus d’une souche de *M. leprae* ou une hétéro résistance de souche, si au moins une des souches présente une mutation. Dans ce cas, une des sondes mutées ainsi que le type sauvage correspondant peuvent apparaître.

Tableau 3. Mutations détectées par le kit GenoType LepraeDR pour les gènes *rpoB*, *gyrA* et *folP1*.

► Mutations au niveau du gène *rpoB* et bandes sauvages et mutées correspondantes

| Bande type sauvage manquante | Codons analysés | Bande mutation en développement | Mutation |
|------------------------------|-----------------|---------------------------------|---|
| <i>rpoB</i> WT1 | 429-434 | | G432S |
| <i>rpoB</i> WT2 | 437-442 | | Q438V F439/M440 KF insertion* D441N* D441Y* |
| <i>rpoB</i> WT3 | 449-455 | <i>rpoB</i> MUT1 | H451Y H451D H451V* |
| <i>rpoB</i> WT4 | 455-459 | <i>rpoB</i> MUT2 | S456L S456M S456F S456W* L458V* L458P* |

* Ces mutations rares ont été détectée uniquement théoriquement (in silico).

► Mutations au niveau du gène *gyrA* et bandes sauvages et mutées correspondantes (numération selon le génome *M. leprae*).

| Bande type sauvage manquante | Codons analysés | Bande mutation en développement | Mutation |
|------------------------------|-----------------|---------------------------------|---------------|
| <i>gyrA</i> WT | 89 91 | <i>gyrA</i> MUT | G89C* A91V |

* Cette mutation rare a été détectée uniquement théoriquement (in silico).

► Mutations au niveau du gène *folP1* et bandes sauvages et mutées correspondantes (numération selon le génome *M. leprae*)

| Bande type sauvage manquante | Codons analysés | Bande mutation en développement | Mutation |
|------------------------------|-----------------|---------------------------------|----------------------|
| <i>folP1</i> WT | 53 | | T53A T53R T53I |
| | 55 | <i>folP1</i> MUT | P55L P55R |

3.5. Contribution personnelle au travail

En 2016, le CDTLUB de Pobè a mis en place le diagnostic de *M. leprae* par PCR. Le laboratoire a souhaité par la suite proposer un test de détection des résistances aux antilépreux afin d'optimiser la prise en charge des patients.

Dans ce cadre, j'ai eu comme objectifs, dans un premier temps, de tester le kit GenoType Leprae DR au laboratoire de bactériologie du CHU d'Angers à partir de prélèvements positifs adressés par le CDTLUB de Pobè et, dans un deuxième temps, de rédiger une procédure

complète permettant au laboratoire du CDTLUB de Pobè de mettre en place ce test et de pouvoir en interpréter les résultats.

3.5.1. Matériels et méthodes

Un total de 12 extraits d'ADN positifs à *M. leprae* provenant de 12 prélèvements de patients ayant consulté au CDTLUB de Pobè ont été adressés au laboratoire de bactériologie du CHU d'Angers afin de rechercher des résistances à la rifampicine, à l'ofloxacine et à la dapsonne, avec le kit GenoType LepraeDR, les patients n'ont été jamais traités contre la lèpre.

Les prélèvements analysés étaient du suc dermique d'oreille, des écouvillons de narine et un écouvillon cutané. Au CDTLUB de Pobè a été réalisé un examen direct par coloration de Ziehl ainsi qu'une PCR en temps réel ciblant le gène RLEP.

Les caractéristiques des 12 extraits d'ADN positifs analysés par le laboratoire de bactériologie du CHU d'Angers sont présentées dans le **Tableau 4**.

Tableau 4 : Caractéristiques des 12 échantillons adressés par le CDTLUB de Pobè.

| Numéro d'échantillon | Type de prélèvement (Site) | Résultat coloration Ziehl-Neelsen | Quantification bactérienne (unité génome/ml) |
|----------------------|----------------------------|-----------------------------------|--|
| 3/21 | Suc dermique (Oreilles) | - | $3,4.10^3$ |
| 5/21 | Suc dermique (Oreilles) | 5+ | $2,8.10^7$ |
| 670a/21 | Suc dermique (Oreilles) | 4+ | $9,7.10^5$ |
| 670b/21 | Écouvillon (Dos) | 4+ | $4,1.10^5$ |
| 810a/21 | Suc dermique (Oreilles) | - | $2,5.10^4$ |
| 1079b/21 | Ecouvillon (Narines) | - | $1,0.10^7$ |
| 1094a/21 | Suc dermique (Oreilles) | 4+ | $1,3.10^6$ |
| 1094b/21 | Ecouvillon (Narines) | 3+ | $6,4.10^7$ |
| 0058/22 | Suc dermique (Oreilles) | - | $4,5.10^2$ |
| 101b/22 | Écouvillon (Narines) | 1+ | $1,8.10^5$ |
| 101a/22 | Suc dermique (Oreilles) | 1+ | $7,8.10^4$ |
| 139/22 | Suc dermique (Oreilles) | 6+ | $2,0.10^7$ |

Légende : - : Absence ; 1+ : 1 à 10 bacilles/ 100 champs ; 2+ : 1 à 10 bacilles/ 10 champs ; 3+ : 1 à 10 bacilles/ champ ; 4+ : 10 à 100 bacilles/ champs ; 5+ : 100 à 1000 bacilles/ champs ; 6+ : > 1000 bacilles/ champs.

L'identification génétique des résistances de *M. leprae* à la rifampicine, l'ofloxacine et à la dapsonne a ensuite été réalisée par le kit GenoType LepraeDR, selon les recommandations

du fournisseur. L'étape d'extraction n'a pas été réalisée étant donné que l'ADN adressé au laboratoire par le CDTLUB de Pobè était déjà extrait.

Les différentes étapes réalisées par le kit GenoType LepraeDR sont présentées dans l'**Annexe 1** et décrites brièvement ci-dessous.

a) Etape d'amplification d'ADN à l'aide d'amorces biotinylées

Le mélange réactionnel, correspondant à 45µl par échantillon à tester, a été préparé dans une pièce exempte d'ADN contaminant selon le protocole présenté dans le **Tableau 5**.

Tableau 5 : Composition du mélange réactionnel permettant de réaliser l'étape d'amplification d'ADN avec le kit GenoType LepraeDR.

| Réactif | Volume pour 1 tube |
|---|--------------------|
| MIX GenoType LepraeDR PNM (nucléotides+ amorces biotinylées, fourni par le kit) | 35 µl |
| Taq polymérase AmpliTaq Gold (Applied) 5U/µL | 0,2 µl |
| Tampon de PCR 10x pour AmpliTaq Gold (Applied) | 5 µl |
| MgCl ₂ 25mM | 2 µl |
| Eau pour PCR Invitrogen | 2,8 µl |

Un volume de 5µl d'ADN extrait est ensuite ajouté, dans une zone séparée et la PCR réalisée selon le protocole suivant :

| | |
|---|-------------|
| 15 min 95°C | 1 cycle |
| 30 sec 95°C 2 min 58°C | } 10 cycles |
| 25 sec 95°C 40 sec 53°C 40 sec 70°C | |
| 8 min 70°C | 1 cycle |

b) Etape d'hybridation.

Chacune de ces étapes est détaillée dans l'**Annexe 1**. Le principe technique a été décrit dans la partie 3.4.1. du paragraphe « le kit GenoType LepraeDR pour la détection de la résistance aux antilépreux »

c) Evaluation et interprétation des résultats.

Cette étape a été décrite dans la partie 3.4.2. du paragraphe « le kit GenoType LepraeDR pour la détection de la résistance aux antilépreux ».

d) Confirmation des mutations par séquençage Sanger.

Les mutations détectées par la bandelette ont été confirmées par séquençage de type Sanger. Les amorces utilisées ont été celles publiées par l'OMS (50).

Pour le séquençage du gène *foIP1*, les amorces utilisées étaient : OMS_foIP1_F: CTTGATCCTGACGATGCTGT et OMS_foIP1_R: CCACCAGACACATCGTTGAC. Concernant le séquençage du gène *gyrA*, les amorces utilisées étaient : OMS_gyrA_F: ATG GTC TCA AAC CGG TAC AT C et OMS_gyrA_R: TAC CCG GCG AAC CGA AAT TG.

Le déroulé des différentes étapes de séquençage a été le suivant :

► Réalisation de la PCR permettant d'amplifier les séquences d'intérêt

Le mélange réactionnel a été préparé selon le protocole présenté dans le **Tableau 6**.

Tableau 6 : Composition du mélange réactionnel permettant de réaliser l'étape d'amplification d'ADN.

| Réactifs | Volume pour 1 tube |
|------------------------------------|--------------------|
| Tampon 5X GoTaq FlexiBuffer | 10 µl |
| MgCl ₂ 25mM | 3 µl |
| dNTP (5mM chaque) | 2 µl |
| Eau pour PCR (Invitrogen) | 21,75 µl |
| OMS_Amorce_F 20pmol/µl | 1,5 µl |
| OMS_Amorce_R 20pmol/µl | 1,5 µl |
| GoTaq G2Flexi DNA polymerase 5U/µL | 0,25 µl |

Ensuite, 10 μL de chaque extrait d'échantillon sont ajoutés et la PCR réalisée selon le profil suivant (**Tableau 7**) :

Tableau 7 : Profil d'amplification de la PCR permettant le séquençage des gènes d'intérêt.

| Température | Durée | Nombre de cycles |
|-------------|--------|------------------|
| 95°C | 2 min | 1 |
| 95°C | 30 s | |
| 58°C | 30 s | 40 |
| 72°C | 1 min | |
| 72°C | 5 min | 1 |
| 4°C | infini | |

► Purification des amplicons

Cette étape permet d'éliminer tous les réactifs afin de ne garder que les amplicons.

► Réalisation de la PCR de séquence

Le mélange réactionnel a été préparé selon le protocole présenté dans le **Tableau 8**.

Tableau 8 : Composition du mélange réactionnel permettant de réaliser l'étape la PCR de séquence.

| Réactifs | Volume pour 1 tube |
|---|--------------------|
| Tampon 5 Sequencing buffer BigDye terminator V3.1 | 3 μl |
| OMS_Amorce_R ou OMS_Amorce_F (20pmol/ μl) | 0,5 μl |
| BigDye terminator V3.1 | 1 μl |
| Eau pour PCR (Invitrogen) | 10,5 μl |

Ensuite, 5 μL d'amplicons précédemment purifiés sont ajoutés et la PCR réalisée selon le profil suivant (**Tableau 9**) :

Tableau 9 : Profil d'amplification de la PCR de séquence.

| Température | Durée | Nombre de cycles |
|-------------|-------|------------------|
| 96°C | 1 min | 1 |

| | | |
|------|--------|----|
| 96°C | 20 s | |
| 50°C | 5 s | 25 |
| 60°C | 4 min | |
| 4°C | infini | |

► Séquençage de l'ADN

Après purification des amplicons obtenus lors de la réaction de séquence, le séquençage de la séquence d'intérêt est réalisé à l'aide du séquenceur 3130xl Genetic Analyzer de PerkinElmer, selon le protocole du fournisseur.

► Analyse des séquences

Un « nettoyage » des séquences est ensuite réalisé à l'aide du logiciel ChromasPro v1.34. Ce logiciel permet d'aligner la séquence obtenue avec l'amorce OMS_Amorce_F, avec celle obtenue avec l'amorce OMS_Amorce_R, pour chaque échantillon analysé et de former une séquence consensus. Cette séquence consensus est ensuite analysée à l'aide du logiciel Clustal X2 avec le gène de référence, dans le but d'identifier la/les mutation(s).

3.5.2. Résultats

Parmi les 12 prélèvements positifs à *M. leprae* par PCR RLEP adressés par le CDTLUB de Pobè au laboratoire de bactériologie du CHU d'Angers, 8 présentaient une coloration de Ziehl Neelsen positive (**Tableau 4**). Le **Tableau 10** détaille les résultats des bandelettes pour chacun des 12 prélèvements. La **figure de l'Annexe 2** représente les résultats obtenus.

Tableau 10 : Résultat du test GenoType LepraeDR sur les 12 échantillons adressés par le CDTLUB de Pobè.

| Echantillon | Résultat par bande | | | | | | | | | | | | | | | |
|------------------------------------|--------------------|----|----|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|--------------|--------------|--------------|
| | CC | UC | ML | <i>rpoB</i> | <i>rpoB</i> | <i>rpoB</i> | <i>rpoB</i> | <i>rpoB</i> | <i>rpoB</i> | <i>rpoB</i> | <i>gyrA</i> | <i>gyrA</i> | <i>gyrA</i> | <i>folP1</i> | <i>folP1</i> | <i>folP1</i> |
| | | | | | WT1 | WT2 | WT3 | WT4 | MUT1 | MUT2 | <i>gyrA</i> | WT | MUT | <i>folP1</i> | WT | MUT |
| 3/21 - Suc dermique (Oreilles) | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5/21 - Suc dermique (Oreilles) | + | + | + | + | + | + | + | + | - | - | + | + | - | + | - | - |
| 670a/21 - Suc dermique (Oreilles) | + | + | + | + | + | + | + | + | - | - | + | + | - | + | + | - |
| 670b/21 - Ecouvillon (Dos) | + | + | + | + | + | + | + | + | - | - | + | + | - | + | + | - |
| 810a/21 - Suc dermique (Oreilles) | + | + | + | + | + | + | + | + | - | - | + | + | + | + | + | - |
| 1079b/21 - Ecouvillon (Narines) | + | + | + | + | + | + | + | + | - | - | + | + | - | + | + | - |
| 1094a/21 - Suc dermique (Oreilles) | + | + | + | + | + | + | + | + | - | - | + | + | - | + | + | - |
| 1094b/21 - Ecouvillon (Narines) | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 0058/22 - Suc dermique (Oreilles) | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 101b/22 - Ecouvillon (Narines) | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 101a/22 - Suc dermique (Oreilles) | + | + | + | + | + | + | + | + | - | - | + | + | - | + | + | - |
| 139/22 - Suc dermique (Oreilles) | + | + | + | + | + | + | + | + | - | - | + | + | - | + | + | - |

CC : Contrôle Conjugué ; UC : Contrôle Universel ; ML : *Mycobacterium leprae* ; WT : sonde type sauvage ; MUT : sonde mutation.

Le test GenoType LepraeDR n'a pas mis en évidence d'ADN de *M. leprae* pour quatre des prélèvements (absence de la bande « ML »). Il s'agit des prélèvements 3/21, 1094b/21, 0058/22 et 101b/22 (Tableau 10).

Le test GenoType LepraeDR a par ailleurs montré un profil de sensibilité à la rifampicine pour tous les prélèvements positifs à *M. leprae*. Concernant la sensibilité aux fluoroquinolones, tous les prélèvements positifs à *M. leprae* étaient sensibles, excepté le prélèvement 810a/21 qui a mis en évidence une hétéro-résistance (S/R) objectivée par la présence concomitante d'une bande au niveau de la sonde *gyrA* WT et *gyrA* MUT, d'intensité quasi égale (Figure 4).

Enfin, le test GenoType LepraeDR a montré que tous les prélèvements étaient sensibles à la dapsones excepté le prélèvement 5/21 qui a montré une absence de bande à la fois pour la sonde *folP1* WT et *folP1* MUT, suggérant une résistance non détectable par le test (Figure 4).

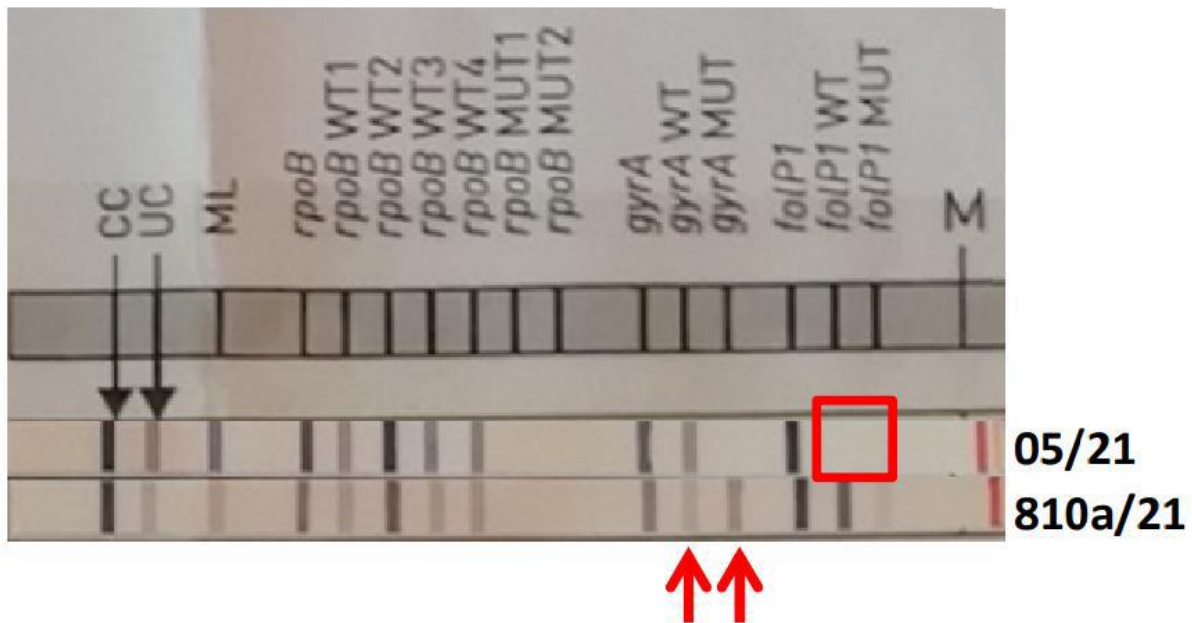


Figure 4: Résultat du test GenoType LepraeDR sur les prélèvements 05/21 et 810a/21.

La résistance aux fluoroquinolones de *M. leprae* présent dans le prélèvement 810a/21 ainsi que la résistance à la dapsones de *M. leprae* présent dans le prélèvement 05/21

ont été confirmées par séquençage respectivement du gène *gyrA* et *folP1*, afin de valider les résultats obtenus par le kit GenoType LepraDR.

Le séquençage du gène *folP1* de *M. leprae* présent dans le prélèvement 5/21 a permis d'obtenir une séquence de 251 pb. L'alignement avec la séquence standard publiée par l'OMS (50) met en évidence une substitution du nucléotide A par le nucléotide G à la position 157, ce qui correspond au codon 53 du gène (**Figure 5**). Il s'agit ici d'une substitution de l'acide aminé thréonine par l'alanine au niveau de la position 53 de la protéine (Thr53Ala). Cette mutation n'est pas détectable par le kit, expliquant l'absence de bande pour *folP1MUT* en plus de l'absence de bande *folP1WT*. Cette mutation est déjà décrite dans la littérature, elle occupe le troisième rang en termes de fréquence de mutation conférant la résistance à la dapsonne chez *M. leprae* (51).

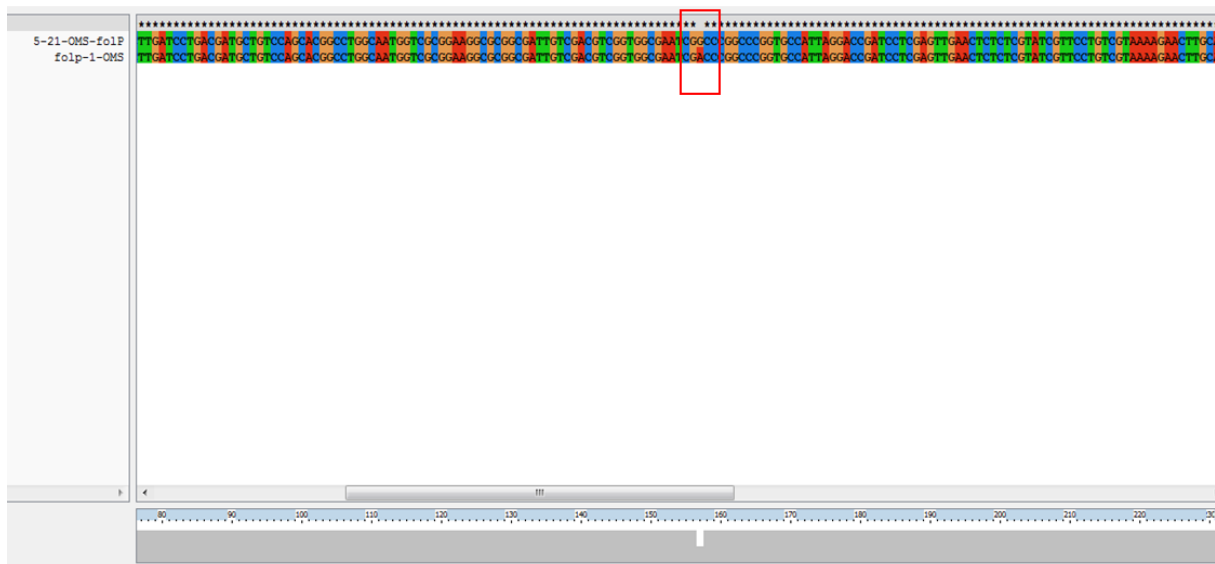


Figure 5. Résultat de l'alignement de la séquence *folP1* obtenue par séquençage Sanger à partir du prélèvement 5/21, avec le gène *folP1* standard publié par l'OMS.

Le séquençage du gène *gyrA* de *M. leprae* présent dans le prélèvement 810a/21 a mis en évidence un double pic au niveau du nucléotide 91, avec la présence d'un nucléotide G dont le pic est majoritaire, et d'un nucléotide A, dont le pic est minoritaire (**Figure 6**). Le nucléotide G confère le caractère sensible aux fluoroquinolones et le nucléotide A, le caractère résistant. Le remplacement du nucléotide G par A modifie la composition en

acides aminés de la protéine. Ainsi, l'alanine est remplacée par une valine (mutation Ala91Val, détecté par la bandelette).

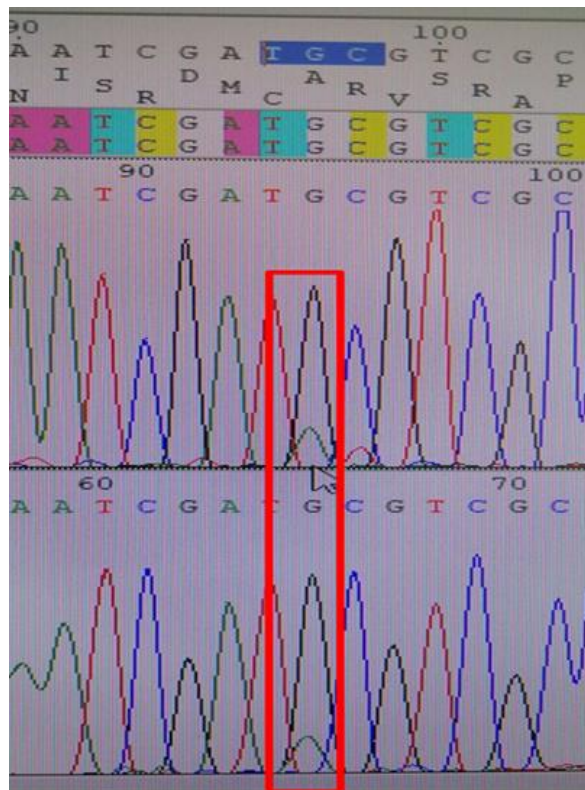


Figure 6. Résultats du chromatogramme montrant la présence de 2 pics concomitants.

3.5.3. Discussion des résultats obtenus

Le kit GenoType LepraeDR permettant l'identification génétique des résistances aux trois antilépreux majeurs (rifampicine, ofloxacine, dapsonne) a été appliqué dans le but de proposer un test rapide et aisé d'utilisation dans les pays où l'accès au séquençage n'est pas possible. Le kit est recommandé à partir d'ADN extraits de biopsies cutanées positives à la coloration de Ziehl Neelsen.

Dans notre étude, parmi les 12 prélèvements analysés, 4 étaient négatifs à la coloration de Ziehl. Ces prélèvements avaient toutefois été analysés avec le kit car la PCR RLEP, réalisé au CDTLUB de Pobè, avait confirmé la présence d'ADN de *M. Leprae*. Pour 2 d'entre eux, le kit GenoType LepraeDR n'a pas pu être interprété du fait d'une absence de bande au niveau du témoin ML, en lien avec une faible quantité d'ADN de *M. leprae*

quantifiée lors de la PCR RLEP ($3,4.10^3$ et $4,5.10^2$ UG/ml). Concernant les deux autres prélèvements négatifs à la coloration de Ziehl et analysés avec le kit GenoType LepraeDR, les résultats ont pu être interprétés (tous les témoins étaient valides) ; un extrait d'ADN de prélèvement d'écouvillon de narine ne présentait pas de mutation pour les gènes recherchés mais pour l'extrait d'ADN du prélèvement 810a/21 (suc dermique d'oreille), nous avons trouvé une mutation au niveau du gène *gyrA*.

Parallèlement, 2 extraits d'ADN dont les prélèvements ont été positifs à la coloration de Ziehl Neelsen n'ont pas pu être interprétés avec le kit GenoType LepraeDR, certains des contrôles n'ayant pas été validés. Les deux prélèvements concernés étaient des écouvillons de narine et pour l'un d'entre eux, la concentration bactérienne était importante, estimée à $6,4.10^7$ UG/ml lors de la PCR RLEP. Le test GenoType LepraeDR avait été réalisé une seconde fois sur ce même extrait d'ADN qui a confirmé les résultats du premier test. Il est possible, notamment pour ce dernier prélèvement, que l'ADN ait été dégradé durant le processus de conservation, de transport et d'envoi au laboratoire de bactériologie du CHU d'Angers, expliquant un test GenoType LepraeDR négatif alors que la quantité d'ADN de *M. leprae* était importante.

Selon les préconisations du kit GenoType LepraeDR, seules les biopsies cutanées sont recommandées pour l'analyse génétique des résistances aux antilépreux. Dans notre étude, parmi les 12 prélèvements analysés, 8 correspondaient à du suc dermique d'oreille, 3 à des écouvillonnages de narine et 1 à un écouvillonnage cutané au niveau du dos et nous avons pu interpréter le test pour 6 des 8 prélèvements de suc dermique d'oreille, pour 2 des 3 écouvillonnages de narine et pour le prélèvement d'écouvillonnage cutané du dos. Plusieurs études ont montré l'intérêt de l'écouvillonnage de la narine pour réaliser le diagnostic de la lèpre (52,53). En effet, la charge bactérienne peut être importante au niveau de cette localisation anatomique. Il a aussi été montré que le suc dermique au niveau du lobe des oreilles pouvait être un bon prélèvement pour le diagnostic de la lèpre (54).

Le nombre de prélèvements analysés dans notre étude est bien trop faible pour tirer des conclusions quant aux types de prélèvements pouvant être étudiés avec le kit GenoType LepraeDR, toutefois, nos résultats sont plutôt encourageants. Il serait intéressant à présent de réaliser une étude de plus grande ampleur testant des prélèvements de différente nature.

Le kit GenoType LepraeDR peut présenter quelques limites. Notamment, il ne détecte que certaines mutations sur les gènes *rpoB*, *gyrA* et *foIP*, celles le plus fréquemment retrouvées. Le cas du prélèvement 5/21, négatif pour la bande sauvage et mutée du gène *foIP* a montré la présence d'une souche résistante à la dapsonne, mais dont la mutation n'est

pas connue du test. Afin de connaître le nucléotide muté, nous avons séquencé le gène. Dans notre cas, nous avons mis en évidence une substitution du nucléotide A par le nucléotide G à la position 157, conduisant à une substitution d'un acide aminé Thr53Ala. Cette mutation a déjà été décrite par Kai *et al.* en 1999 (51). Enfin, selon les données publiées par Cambau *et al.* en 2018, seules 4 mutations conférant une résistance à la dapsonne ont été décrites au Bénin entre 2009 et 2015 (37).

3.5.4 Conclusion

Nous avons appliqué la technique GenoType LeptraeDR à partir des extraits d'ADN de prélèvements positifs à *M. leprae* adressés par le CDTLUB de Pobè. Un protocole détaillé a été écrit et adressé au laboratoire de biologie moléculaire du CDTLUB de Pobè, accompagné de tout le matériel nécessaire pour la mise en place de ce test. Depuis fin février 2023, le CDTLUB de Pobè a ainsi la possibilité de proposer l'identification génétique des résistances de *M. leprae* à la rifampicine, à l'ofloxacine et à la dapsonne aux cliniciens qui prennent en charge les patients atteints de lèpre. La mise en place de cette technique est une grande avancée dans la prise en charge globale et intégrée des patients atteints de MTN-C.

DISCUSSION GÉNÉRALE ET CONCLUSION

En raison des complications physiques et des stigmatisations sociales importantes qu'elles génèrent, les MTN-C représentent un lourd fardeau à l'égard des pays concernés. Pour combattre ces maladies, une prise en charge individuelle des patients est souvent nécessaire. De plus, afin d'éviter les complications et les récives, il est indispensable de disposer de moyens financiers suffisants, de structures de soins, de matériel adéquat et de personnel de santé bien formé. Enfin, les patients et les proches aidants doivent être acteurs de la prise en charge.

Le Bénin a été parmi les premiers pays à déployer un plan quinquennal (2012-2016) de lutte intégrée contre les MTN-C en renforçant la surveillance globale et en améliorant la coordination entre les différents partenaires impliqués dans la prise en charge de ces maladies. Le contexte politique et économique favorable du pays a permis de mettre en place les différents axes de ce plan pour atteindre les objectifs (18). Ainsi, l'instauration d'un plan directeur de lutte intégrée contre les MTN (via la création du PNLLUB), de même que le développement de structures de dépistage et de prise en charge de ces maladies (via les 4 CDTLUB) ont rencontré un grand succès sur le terrain. Quelques fois même, l'application de protocoles a précédé les recommandations de l'OMS.

Les consultations foraines avec des équipes mobiles pour le dépistage intégré des MTN-C et des autres maladies de la peau ont été organisées dans les villages au plus près des patients. Elles ont montré un rôle majeur dans la prise en charge des cas. Les équipes médicales ont ainsi pu pallier la méconnaissance des patients et des proches aidants s'agissant des symptômes de l'ulcère de Buruli et des autres MTN-C, et proposé un suivi médical personnalisé. De plus, étant donné que ces consultations ont été mises en place avec l'accord et l'aide de la communauté des villageois (chef du village, religieux, instituteurs,...), l'adhésion de la population a été excellente.

Nous avons pu observer que le système de santé du pays était en capacité d'évoluer avec la situation sur le terrain pour assurer la couverture la plus large possible des MTN-C. En effet, l'étude de l'évolution du statut épidémiologique de l'ulcère de Buruli, de la lèpre, de la filariose lymphatique et de l'onchocercose, connus pour être endémiques au Bénin depuis longtemps, a montré une baisse importante de leur fardeau, confirmant l'efficacité des stratégies de lutte appliquées par le système de santé du pays. L'analyse de la situation épidémiologique d'autres MTN-C a également pu montrer une sous-estimation de certaines d'entre elles, comme par exemple la gale. En effet, 88,4% des 646 patients présentant une MTN-C étaient diagnostiqués avec une gale. Ce constat a conduit à alerter les autorités sanitaires locales et le ministère de

santé du pays afin que des stratégies de lutte contre ce parasite soient mises en place pour assurer une prise en charge adéquate des patients affectés par cette pathologie.

Le laboratoire du CDTLUB de Pobè est devenu laboratoire expert dans le diagnostic de l'ulcère de Buruli mais également de la lèpre. Il a su par ailleurs proposer avec beaucoup de réactivité des tests qPCR pour le diagnostic COVID lors de l'épidémie mondiale. Ces activités ont pu être déployées à la faveur du soutien d'organisations gouvernementales et non gouvernementales telles que la fondation Raoul Follereau ou encore la fondation ANESVAD qui assurent les ressources financières de différents projets. Grâce aux expertises acquises, le laboratoire du CDTLUB de Pobè a également été moteur dans la mise en place du BU-LABNET, un solide réseau de 11 laboratoires de 9 pays africains endémiques pour l'ulcère de Buruli (Bénin, Cameroun, Côte d'Ivoire, Ghana, République Démocratique du Congo, Togo, Libéria, Nigéria, Gabon). Ce réseau s'est engagé à améliorer la qualité du diagnostic moléculaire de l'ulcère de Buruli par l'utilisation de procédures standardisées et par l'implication dans un programme d'assurance qualité externe. Le réseau BU-LABNET a également vocation à partager des connaissances entre les laboratoires membres, à fournir une formation et un soutien à tous les laboratoires souhaitant développer la PCR *M. ulcerans* et à améliorer la qualité des données communiquées par l'OMS en Afrique (27).

Des collaborations entre le CDTLUB de Pobè et l'équipe de recherche d'Angers ont été initiées au début des années 2000 pour étudier l'écologie de *M. ulcerans* et son mode de transmission à l'Homme. Rapidement, le laboratoire de bactériologie du CHU d'Angers a rejoint les deux équipes et a proposé la PCR *M. ulcerans* pour confirmer les suspicions cliniques d'ulcère de Buruli dépistées au CDTLUB. Devant la volonté du CDTLUB d'acquérir de l'autonomie dans le diagnostic biologique de l'ulcère de Buruli et de permettre une prise en charge plus rapide des patients atteints, la plateforme de biologie moléculaire a été créée avec l'aide technique des équipes d'Angers. Ces collaborations, qui ont su se pérenniser dans le temps ont ainsi permis de développer d'autres axes de recherche comme l'étude de la réponse de l'hôte au cours des différentes phases de colonisation du tissu cutané ou encore l'étude de l'écologie de la bactérie (55,56,57,58,59,60,61).

Plusieurs obstacles persistent cependant au Bénin s'agissant de la lutte contre les MTN-C et ils constituent un véritable challenge pour le pays.

Certaines des MTN-C sont co-endémiques dans le pays et elles partagent des signes cutanés similaires rendant le diagnostic clinique souvent difficile. De plus, les signes cliniques retrouvés dans ces infections sont souvent similaires à ceux observés dans d'autres pathologies cutanées comme les plaies chroniques qui sont également fréquemment diagnostiquées chez les patients. Pour une prise en charge optimale des cas, il est nécessaire d'avoir recours à des dermatologues. Malheureusement, au Bénin, cette spécialité médicale est très récente. En outre, peu de médecins se formant à la dermatologie s'installent en milieu urbain en clinique privée. Très peu de dermatologues sont présents dans les zones rurales où résident la majorité des patients atteints de MTN-C. Des équipes d'infirmiers et d'aides-soignants ont été déployées pour pallier le manque de dermatologues mais elles ne disposent pas de toutes les compétences médicales. De surcroît, elles souffrent d'un manque de matériel nécessaire à la prise en charge des patients dans les centres de santé. Ces équipes sont également souvent confrontées à la présence de « faux soignants » qui prennent en charge les patients sans aucune compétence dans le domaine des pathologies cutanées. Au CDTLUB de Pobè, l'équipe médicale est composée d'un médecin dermatologue en plus d'un médecin de spécialité générale et d'un chirurgien, permettant de répondre au mieux à la prise en charge des patients venant consulter. Les collaborations pérennes entre le CDTLUB de Pobè et Angers ont permis de mettre en place depuis quelques années déjà un partenariat entre le service de dermatologie du CHU d'Angers et les acteurs sur le terrain de Pobè avec la réalisation de missions de la part d'internes et de médecins d'Angers pour sensibiliser les personnels soignants à la reconnaissance des pathologies cutanées.

Une autre problématique à laquelle se heurtent les personnels soignants est la perception culturelle de la maladie. L'ulcère de Buruli en particulier est vu comme relevant de la sorcellerie. Il est dans l'imaginaire collectif un malheur d'origine invisible qui serait l'œuvre des sorciers (62) et sa guérison est spirituelle étant donné que le patient a été « envouté », qu'un « sort » lui a été jeté. Aussi, seule la prière pourrait délivrer l'âme de l'emprise maléfique (62) ou encore une séance chez le tradi-praticien pour se faire désenvouter. De surcroît, de nombreuses personnes estiment que l'ulcère de Buruli est une maladie d'origine africaine et que la médecine d'origine occidentale ne serait pas compétente. Il est d'usage dans les villages de pratiquer l'automédication en utilisant par exemple des cataplasmes à base d'argile ou d'autres composés à appliquer sur la plaie, retardant malheureusement d'autant la prise en charge optimale des patients. Dans notre travail nous avons pu montrer que le succès des consultations foraines a pu être attribué en partie à la communication et aux échanges

d'informations entre les équipes médicales et les différents acteurs ayant autorité dans les villages, tels que les chefs religieux, les élus locaux, les gouvernants, ou encore les chefs de village.

L'accès aux médicaments pour traiter certaines pathologies est également un frein à la guérison des malades. Nous avons montré dans notre étude que la gale était la première MTN-C en termes de taux de détection dans les départements du Plateau et de Ouémé, loin devant l'ulcère de Buruli ou encore la lèpre. La gale est très contagieuse, des épidémies sont décrites, en particulier chez les enfants qui se contaminent à l'école. Pour soigner les patients, un traitement de masse par ivermectine est recommandé par l'OMS. Malheureusement, cette molécule est réservée au Bénin dans le traitement de l'onchocercose et les patients atteints de gale n'ont pas accès à cet anti-parasitaire.

Enfin, la mise en place des différents plans de lutte contre les MTN-C, la surveillance de ces maladies, l'organisation et la logistique des consultations foraines, la formation des personnels médicaux et para-médicaux, exige un financement important et récurrent. Actuellement, les organisations non gouvernementales apportent un soutien financier conséquent mais uniquement pour des missions spécifiques. Les financements n'étant pas pérennes, le déploiement de stratégies à moyen et long terme peut s'avérer compliqué.

En conclusion, ce travail de thèse de doctorat a permis de bien décrire la situation du Bénin vis-à-vis des MTN-C et les problèmes rencontrés par les équipes sur le terrain pour appliquer les stratégies de lutte. Il a également permis de tirer la sonnette d'alarme sur les MTN-C sous-estimées dans le pays et qui constituent un problème important de santé pour la population. Les consultations mobiles ont montré l'importance de réaliser un dépistage intégré de toutes les maladies cutanées fréquentes et pas seulement des MTN-C. Le succès dans la lutte contre les MTN-C et les autres maladies endémiques dépend de l'implication de nombreuses équipes, qu'elles soient médicales, paramédicales ou encore techniques, la multidisciplinarité est ainsi un prérequis. L'organisation mise en place par le CDTLUB de Pobè pour la prise en charge des MTN-C constitue un exemple pionnier qu'il est important de mettre en lumière afin que d'autres pays endémiques à ces maladies puissent bénéficier de cette expérience pour développer des structures efficaces permettant de diminuer le fardeau des MTN-C. Mais le chemin est encore long dans la lutte contre les MTN-C et l'un des axes à approfondir est le

développement d'outils diagnostic simples d'utilisation et peu onéreux pour dépister les patients les plus à risque et vivant dans des contrées loin des centres de soins.

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**ANNEXE 1 : Procédure pour la mise en
place du Kit Genotype LepraeDR au
CDTLUB de Pobè**

| | | |
|---|--|--------------------------------|
|  | PROCEDURE | Version : 1 |
| | Identification génétique des résistances à la rifampicine, à l'ofloxacin et à la dapson de <i>Mycobacterium leprae</i> à l'aide du test GenoType LepraeDR (HAIN LIFESCIENCE) | Date d'application : Mars 2022 |

I. OBJET

La lèpre est une maladie chronique causée par le bacille *Mycobacterium leprae* (*M. leprae*). Elle touche principalement la peau, les nerfs périphériques, la muqueuse des voies respiratoires supérieures ainsi que les yeux. Faute de traitement, elle peut entraîner des lésions progressives et permanentes de la peau, des nerfs, des membres et des yeux.

Le GenoType LepraeDR (VER 1.0 - HAIN LIFESCIENCE) est un test d'identification génétique de *M. leprae* et des résistances à la rifampicine, à l'ofloxacin et à la dapson.

II. DOMAINE D'APPLICATION

Le GenoType LepraeDR (VER 1.0) est un test qualitatif de diagnostic *in vitro* pour l'identification de *M. leprae* et la détermination de sa résistance à la rifampicine et/ou l'ofloxacin et/ou la dapson. Ce test sera réalisé au CDTLUB de Pobè, à partir d'extraits d'ADN de prélèvements positifs à *M. leprae* par qPCR ciblant le gène RLEP.

La présence de *M. leprae* est déterminée par la détection de la séquence répétée RLEP.

La résistance à la rifampicine est déterminée par la détection des principales mutations du gène *rpoB*, codant pour la sous-unité b de l'ARN polymérase.

La résistance à l'ofloxacin ou aux autres fluoroquinolones est déterminée par la détection par la principale mutation du gène *gyrA*, codant pour la sous-unité A de l'ADN gyrase

La résistance à la dapson est déterminée par la détection de mutations dans le gène *folP1*, codant pour la dihydroptéroate synthétase

III. DOCUMENTS ASSOCIES

- Notice technique GenoType LepraeDR VER 1.0. Test d'identification génétique des résistances à la Rifampicine, l'Ofloxacin et à la Dapson de *Mycobacterium leprae*.
- Feuille de travail pour PCR multiplex.

IV. PRINCIPE DE LA METHODE

Le test GenoType LepraeDR VER 1.0 est basé sur la technique DNA•STRIP et comporte deux étapes que sont :

étape 1 : amplification multiplex à l'aide d'amorces biotinylées à partir des ADN extraits positifs à *M. leprae*

étape 2 : hybridation inverse sur bandelette

Le Mélange Amorces/Nucléotides (PNM) contient des amorces biotinylées pour l'amplification de régions spécifiques du génome de *M. leprae* (RLEP, *rpoB*, *gyrA* et *folP1*).

Les bandelettes sont recouvertes de sondes spécifiques complémentaires aux acides nucléiques amplifiés. Après dénaturation chimique, les amplicons simple brin formés se lient aux sondes par hybridation. Sous l'action de conditions stringentes, les molécules d'ADN simple brin se lient spécifiquement à leur brin complémentaire, permettant ainsi aux sondes de différencier plusieurs mutations de séquences dans les régions des gènes examinées. Le conjugué streptavidine-phosphatase alcaline est ensuite immobilisé sur la membrane par l'intermédiaire de la biotine présente sur chaque amplicon formé. Enfin, la phosphatase alcaline transforme un substrat ajouté en colorant qui forme un précipité coloré sur la bandelette. Les signaux obtenus sont facilement et rapidement interprétés à l'aide d'une matrice fournie avec chaque kit.

V. ECHANTILLONS

Les prélèvements biologiques sur lesquels le diagnostic de lèpre peut être réalisé sont le suc dermique des oreilles, des écouvillons de narine et des biopsies cutanées. L'ADN est extrait des prélèvements selon la procédure Genolyse mise en place au CDTLUB de Pobè.

Les extraits d'ADN des prélèvements positifs à *M. leprae* sont conservés à -20°C jusqu'à analyse des résistances à la rifampicine, à l'ofloxacine et à la dapsonne à l'aide du test GenoType LeptraeDR.

VI. REACTIFS ET INSTRUMENTS

6.1. Équipements nécessaires

- Thermocycleur pour PCR conventionnelle
- TwinCubator (instrument pour hybridation)
- Congélateur -20°C pour stocker les réactifs propres (dans la pièce « propre »)
- Congélateur -20°C pour stocker les extraits d'ADN (dans la pièce « extraction d'ADN »)
- Congélateur -20°C pour stocker les amplicons (dans la pièce d'amplification)
- Réfrigérateur 2-8°C pour stocker les réactifs propres (dans la pièce « propre »)
- Réfrigérateur 2-8°C pour stocker les extraits d'ADN (dans la pièce « extraction d'ADN »)
- Réfrigérateur 2-8°C pour stocker les réactifs les amplicons (dans la pièce d'amplification)
- Enceinte à flux laminaire pour préparer les mix de PCR
- Micro-centrifugeuse
- Micropipettes ajustables 2 µL, 10 µL, 100 µL, 200 µL et 1000µl
- Vortex
- pipetboy

6.2. Consommables nécessaires

- Gants nitrile
- Microtubes à vis 1,5 mL
- Tubes de 10 – 15 ml pour préparation des solutions d'hybridation
- Embouts de pipettes 2 µL, 10 µL, 100 µL, 200 et 1000 µL stériles et avec filtre

- Pipettes graduées 5 et 10 ml
- Support pour microtubes
- Microtubes pour PCR exempts de contamination par DNase et RNase
- Pinces à usage unique (autant de pinces que de tests à réaliser)
- Papier absorbant
- Feuilles aluminium
- Marqueur indélébile
- Crayon en papier

6.3. Réactifs

6.3.1. Réactifs fournis par le kit GenoType LepraeDR

- Bandelettes sensibilisées avec les sondes spécifiques (LepraeDR VER 1.0 STRIPS)
- Mélange Amorces/Nucléotides (PNM LepraeDR VER 1.0), contenant les amorces spécifiques, les nucléotides et le colorant
- Solution de Dénaturation (DEN)
- Solution d'Hybridation (HYB)
- Solution de Lavage Stringent (STR)
- Solution de Rinçage (RIN)
- Conjugué Concentré (CON-C)
- Tampon Conjugué (CON-D)
- Substrat Concentré (SUB-C)
- Tampon Substrat (SUB-D)
- Bac en plastique blanc permettant de recevoir les bandelettes
- Feuille d'évaluation
- Notice d'utilisation
- Matrice
- Étiquettes des lots

6.3.2. Réactifs nécessaires mais non fournis par le kit GenoType LepraeDR

- ADN polymérase thermostable avec 1 tube Tampon 10X et 1 tube MgCl₂ 25mM
NB : L'ADN polymérase utilisée au laboratoire de bactériologie du CHU d'Angers est l'AmpliTaq Gold™ (Applied, 250U ref N8080241). Le kit comprend également le Tampon 10X ainsi qu'un tube de MgCl₂ 25 mM
- Eau pour préparation injectable (PPI)

6.4. Conservation des réactifs

- Les réactifs fournis par le kit GenoType LepraeDR sont conservés selon les recommandations du fournisseur :

- dès réception, conserver le Mélange Amorces/Nucléotides (PNM) entre 2°C et 8°C dans une zone exempte d'ADN. Si la durée de conservation doit excéder 4 semaines, conserver au congélateur, entre -20°C et -18°C. Éviter les congélations et décongélations répétées du PNM. Lorsque peu d'échantillons sont traités par série, aliquoter le PNM.
- conserver tous les autres composants du kit entre 2°C et 8°C.

- L'eau pour préparation injectable (PPI) est conservée au frigo 2-8°C.

- ADN polymérase, Tampon 10X, MgCl₂ sont conservés selon les recommandations du fournisseur.

- Les ADN extraits de prélèvements positifs à *M. leprae* sont conservés dans le congélateur « extraction d'ADN »

VII AMPLIFICATION

1- Préparer la feuille du travail :

- Enregistrer les noms des patients (ou numéros d'identification)
- Réaliser le plan de plaque (en bas de la feuille de travail)

2- Calculer, à partir de la feuille de travail, le volume de chaque réactif en fonction du nombre d'échantillons + 1 témoin négatif (correspondant à de l'eau PPI) à réaliser

NB : étant donné le coût des réactifs, le mix sera préparé pour « N » tests qui comprennent les échantillons à tester + le témoin négatif (et non pour N+1 tests). Il n'y a pas de témoin positif.

A. Dans la pièce « mix » de PCR :

3- Laisser décongeler le tube Amorces/Nucléotides PNM du kit GenoType LepraeDR, le Tampon 10X et le tube de MgCl₂ du kit AmpliTaq Gold

NB : l'AmpliTaQ Gold™ reste au congélateur

4- Vortexer doucement les réactifs de PCR puis centrifuger

NB : l'AmpliTaQ Gold™ est toujours au congélateur

5- Préparer un tube Eppendorf pour le mix de PCR.

6- Préparer pour chaque échantillon le mix suivant (Annexe 1, feuille de travail) :

- 35 µl du mélange Amorces/Nucléotides PNM
- 5 µl de Tampon 10X
- 2 µl de MgCl₂
- 2,8 µl eau niveau de pureté pour Biologie Moléculaire

NB : préparer le mix pour N échantillons + le témoin négatif

- 7- Recongeler les réactifs
- 8- Vortexer doucement le mix pendant quelques secondes puis centrifuger rapidement pour faire tomber les gouttes
- 9- Sortir l'AmpliTaq Gold™ du congélateur.
10. Ajouter 0,2 µl l'AmpliTaq Gold™ par échantillon au mix
NB : préparer pour N échantillons + le témoin négatif
11. Aspirer et refouler délicatement pour mélanger
12. Recongeler l'AmpliTaq Gold™
13. Homogénéiser le mix par vortex à très faible vitesse puis centrifuger quelques secondes pour faire tomber les gouttes
14. Préparer un portoir couvert de papier aluminium
15. Placer les barrettes pour PCR sur le portoir
NB : autant de tubes que de points de PCR à faire
16. Distribuer 45 µl de mix de PCR dans chaque tube.

B. Sur la paillasse dépôt de l'ADN :

17. Sortir les extraits d'ADN positifs à *M. leprae* à tester du congélateur situé dans la pièce « extraction d'ADN », vortexer et centrifuger.
18. À l'aide d'une pipette, ajouter 5 µl d'ADN extrait ou 5 µl le témoin négatif (correspondant à de l'eau PPI) dans les microtubes pour PCR contenant le mix
19. Bien fermer la barrette, centrifuger quelques secondes pour faire tomber les gouttes.
20. Recongeler les extraits d'ADN
21. Transporter les barrettes pour PCR vers le Thermocycleur.
22. Nettoyer la paillasse du RNase away

C. Dans la pièce du Thermocycleur :

23. Allumer le Thermocycleur

24. Placer les barrettes pour PCR dans le Thermocycleur

25. Fermer le Thermocycleur et choisir le protocole d'amplification suivant, selon les recommandations du fournisseur (durée environ 2h40) :

| TEMPERATURE | DUREE | CYCLE |
|-------------|--------|-------|
| 95° C | 15 min | 1 |
| 95°C | 30 sec | 10 |
| 58°C | 2 min | |
| 95° C | 25 sec | 30 |
| 53°C | 40 sec | |
| 70°C | 40 sec | |
| 70°C | 8 min | 1 |

26. Lancer le Thermocycleur

27. Vérifier la montée de la température jusqu'à atteindre 95°C (première cycle de PCR)

28. Conserver les amplicons au réfrigérateur (situé dans la pièce de PCR) s'ils sont utilisés dans les 24h. Sinon, les conserver au congélateur situé dans la pièce de PCR

VIII. HYBRIDATION

Remarques préalables :

- L'étape d'hybridation est réalisée dans le secteur de post-amplification
- Durant les phases d'incubation, ne pas retourner dans les pièces de PCR pour éviter tout risque de contamination par de l'ADN amplifié
- Bien refermer la poubelle après chaque étape
- A la fin de l'étape, rajouter de la soude dans la poubelle
- le TwinCubator indique la fin de chaque étape par une sonnerie. Appuyer sur la flèche à droite afin de mettre l'appareil en pause, ce qui ne modifie pas la température, le temps de réaliser les gestes techniques préconisés (ajout ou élimination de réactif).

1- Décongeler éventuellement les produits de PCR s'ils ont été congelés préalablement

2- Mettre en marche le TwinCubator :

- sélectionner « PROGRAM »-HYB-5 puis RUN.
- lancer le programme avec la flèche à droite puis réappuyer immédiatement sur la flèche pour le mettre en pause, le programme ne se lancera pas mais la température atteindra 45°C.

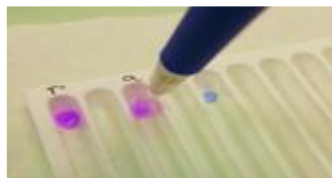
3- Préchauffer les solutions HYB (tampon d'hybridation, vert) et STR à 37 - 45°C avant utilisation, sur la plaque chauffante du TwinCubator

NB : Les réactifs ne doivent présenter aucun précipité. Si besoin, agiter

- 4- Mettre les autres réactifs, à l'exception des solutions CON-C et SUB-C, à température ambiante.
- 5- Dans un tube approprié, diluer le Conjugué Concentré (CON-C, orange) au 1:100 dans un volume adéquat de tampon CON-D. Par échantillon à tester, mettre 1ml de tampon conjugué (CON-D), ajouter 10µl de conjugué concentré (CON-C), puis bien homogénéiser par retournements.
NB : Préparer CON-C dilué au 1 :100 extemporanément
- 6- Dans un tube approprié, diluer le Substrat Concentré (SUB-C, jaune) au 1:100 dans un volume adéquat de tampon SUB-D. Par échantillon à tester, mettre 1ml de tampon SUB-D), ajouter 10µl de substrat concentré (SUB-C), puis bien homogénéiser par retournements.
NB : Préparer SUB-C dilué au 1 :100 extemporanément
- 7- Sortir le bac blanc en plastique (fourni dans le kit GenoType LepraeDR). Il servira à accueillir les bandelettes.
- 8- Numérototer les emplacements utilisés sur le rebord du bac à l'aide d'un marqueur indélébile fin. Laisser un emplacement vide entre deux emplacements utilisés.
NB : tester au maximum 6 amplicons : 5 patients + le témoin négatif
- 9- Centrifuger rapidement les barrettes de PCR.
- 10- Déposer 20 µl de Solution de Dénaturation (DEN, bleu) à une extrémité de chaque emplacement utilisé.

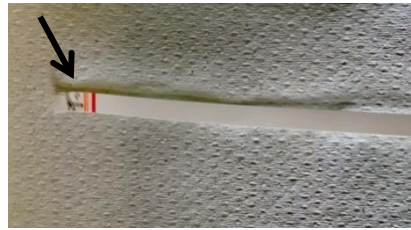


- 11- Ajouter 20 µl d'échantillon amplifié et mélanger les deux solutions par pipetages répétés puis incuber 5 minutes à température ambiante.
NB : il est important de changer de cône entre chaque emplacement



- 12- Pendant ce temps, à l'aide d'une pince, sortir du tube le nombre approprié de bandelettes (STRIPS), les déposer sur une feuille d'aluminium propre.

- 13-** Incrire au crayon en papier le numéro d'identification du patient au-dessus du marquage coloré de chaque bandelette.



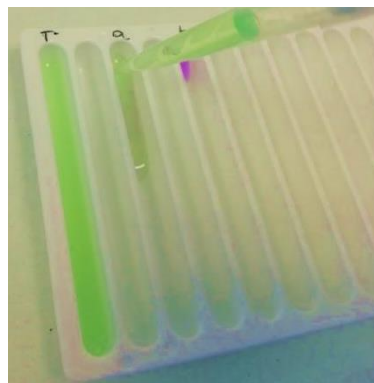
- 14-** A la fin des 5mn d'incubation, ajouter dans chaque emplacement utilisé du bac, 1 ml du Tampon d'Hybridation (HYB, vert) préalablement préchauffé

NB : il est important de changer de cône entre chaque emplacement

- 15-** Homogénéiser doucement par aspirations-refoulements tout le long de chaque emplacement utilisé, jusqu'à ce que le mélange atteigne une coloration homogène verte.

NB : veiller à ne pas faire d'éclaboussures entre les différents emplacements car risque de contamination

NB : il est important de changer de cône entre chaque emplacement



- 16-** A l'aide d'une pince stérile, déposer la bandelette correspondante dans chaque emplacement utilisé. Les bandelettes doivent être entièrement recouvertes par le liquide, avec la face sensibilisée (identifiable par le marquage coloré) tournée vers le haut. Si la bandelette s'oriente mal, la repositionner à l'aide de la pince.

N.B. il est important de changer de pince entre chaque bandelette.



17- Placer le bac dans le bain-marie agitateur du TwinCubator, lancer le programme avec la flèche à droite : incubation 30 minutes sous agitation à 45°C.

18- Puis, à l'aide d'un cône de P1000, aspirer le liquide et le jeter dans la poubelle « liquides ».

NB : il est important de changer de cône entre chaque emplacement

19- Ajouter dans chaque emplacement utilisé 1 ml de Solution de Lavage Stringent (STR, rouge).

NB : il est important de changer de cône entre chaque emplacement



20- Relancer le TwinCubator en appuyant sur la flèche à droite (incubation 15 minutes sous agitation à 45°C).

21- A l'aide d'un cône de P1000, éliminer la Solution de Lavage Stringent.

NB : il est important de changer de cône entre chaque emplacement

22- Ajouter dans chaque emplacement utilisé 1 ml de Solution de Rinçage (RIN).

NB : il est important de changer de cône entre chaque emplacement



23- Relancer le TwinCubator en appuyant sur la flèche à droite (incubation 1 minute sous agitation à 25°C).

24- A l'aide d'un cône de P1000, éliminer la Solution de Rinçage (RIN).

NB : il est important de changer de cône entre chaque emplacement

- 25-** Ajouter dans chaque emplacement utilisé 1 ml de Conjugué CON-C préalablement dilué

NB : il est important de changer de cône entre chaque emplacement



- 26-** Relancer le TwinCubator en appuyant sur la flèche à droite (incubation 30 minutes sous agitation à 25°C).

- 27-** A l'aide d'un cône de P1000, éliminer la solution de Conjugué.

NB : il est important de changer de cône entre chaque emplacement

- 28-** Faire un 1^{er} rinçage : ajouter dans chaque emplacement utilisé 1 ml de Solution de Rinçage (RIN).

NB : il est important de changer de cône entre chaque emplacement

- 29-** Relancer le TwinCubator en appuyant sur la flèche à droite (incubation 1 minute sous agitation à 25°C).

- 30-** A l'aide d'un cône de P1000, éliminer la Solution de Rinçage (RIN).

NB : il est important de changer de cône entre chaque emplacement

- 31-** Faire un 2^{ème} rinçage : ajouter dans chaque emplacement utilisé 1 ml de Solution de Rinçage (RIN).

NB : il est important de changer de cône entre chaque emplacement

- 32-** Relancer le TwinCubator en appuyant sur la flèche à droite (incubation 1 minute sous agitation à 25°C).

- 33-** A l'aide d'un cône de P1000, éliminer la Solution de Rinçage (RIN).

NB : il est important de changer de cône entre chaque emplacement

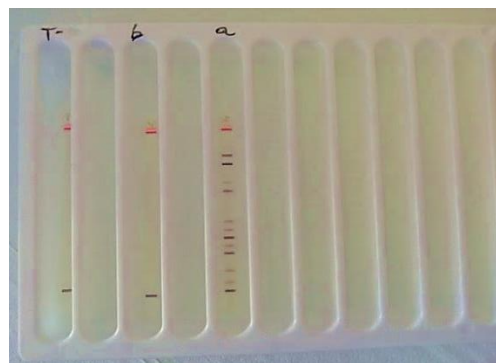
- 34-** Ajouter dans chaque emplacement utilisé 1 ml d'eau PPI.

NB : il est important de changer de cône entre chaque emplacement

- 35-** Relancer le TwinCubator en appuyant sur la flèche à droite (incubation 1 minute sous agitation à 25°C).
- 36-** Eteindre le TwinCubator
- 37-** A l'aide d'un cône de P1000, éliminer totalement l'eau PPI.
NB : il est important de changer de cône entre chaque emplacement
- 38-** Ajouter dans chaque emplacement 1 ml de Substrat *SUB-C* préalablement dilué au 1:100.
NB : il est important de changer de cône entre chaque emplacement
- 39-** Incuber à l'obscurité, sous une feuille d'aluminium, sans agiter.
- 40-** Arrêter la réaction dès que des lignes colorées sont clairement visibles, en rinçant brièvement par deux fois avec 1ml d'eau PPI :
 - à l'aide d'une P1000, éliminer le Substrat *SUB-C* préalablement dilué au 1:100.
 - ajouter à chaque emplacement 1 ml d'eau PPI.
 - à l'aide d'un cône de P1000, éliminer totalement l'eau PPI.
 - ajouter à chaque emplacement 1 ml d'eau PPI.
 - A l'aide d'un cône de P1000, éliminer totalement l'eau PPI.
NB : il est important de changer de cône entre chaque emplacement

Le temps de révélation peut varier en fonction des conditions de température de la pièce (de 3 à 20 mn).

Des temps de révélation trop longs peuvent entraîner un bruit de fond qui peut gêner l'interprétation des résultats



- 41-** Récupérer les bandelettes à l'aide d'une pince stérile et les sécher entre deux feuilles de sopalin.
NB : une même pince stérile peut être utilisée pour toutes les bandelettes
- 42-** Remplir la poubelle avec de la soude, la fermer, la mettre dans le sac autoclave et fermer le sac

- 43- Nettoyer le bac blanc en plastique avec de l'alcool modifié non camphré, rincer puis avec du RNase away, puis rincer à l'eau PPI
- 44- Nettoyer la paillasse et les surfaces de l'appareil à l'aide de détergeant désinfectant.

IX EVALUATION ET INTERPRETATION DES RESULTATS

1. Une feuille d'évaluation et une matrice sont fournies avec le kit.
2. Lors de la première utilisation du kit, coller l'étiquette comportant les références et le numéro de lot du kit sur la feuille d'évaluation.
3. Coller les bandelettes avec scotch dans leur emplacement réservé, en alignant les bandes CC et UC (ou IC) des bandelettes avec les bandes CC et UC (ou IC) de la feuille.
4. Identifier à côté de chaque bandelette l'identité du patient
5. Déterminer le numéro des bandes positives à l'aide de la matrice fournie.
6. Noter les bandes positives dans l'avant-dernière colonne, et déterminer le statut de sensibilité (S) ou de résistance (R) pour chacun des 3 gènes rpoB, gyrA et folp1.
7. Noter le résultat dans la dernière colonne.
8. Les feuilles d'évaluation et les matrices sont rangées à l'abri de la lumière.



ANNEXE 2 :

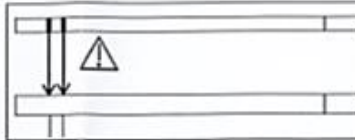
**Résultats des bandelettes des 12
prélèvements analysés par le kit
GenoType LepraeDR.**

GenoType LepraeDR 12

VER 1.0
00320-0817-02-3

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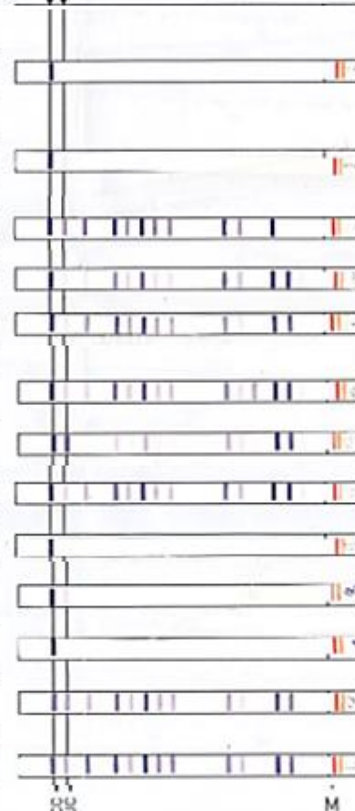


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|----|---------|----------|---------|----------|----------|-----------|-----------|-----------|-----------|
| ML | rpoB WT | rpoB MUT | gyrA WT | gyrA MUT | folP1 WT | folP1 MUT | RMP | FLQ | DAP |
| | | | | | | | sensitive | sensitive | sensitive |
| | | | | | | | resistant | resistant | resistant |
| | | | | | | | sensitive | sensitive | sensitive |
| | | | | | | | resistant | resistant | resistant |



Le 09/19/2021 FF

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| b | 1094b/22 | |
| 8 | 0058/22 | |
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LOT _____
 HYB _____ min
 STR _____ min
 SUB _____ min

ANNEXE 3:

Article 3: Clonal Dissemination of Plasmid-Mediated Carbapenem and Colistin Resistance in Refugees Living in Overcrowded Camps in North Lebanon

Article

Clonal Dissemination of Plasmid-Mediated Carbapenem and Colistin Resistance in Refugees Living in Overcrowded Camps in North Lebanon

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Abstract: Carbapenem and colistin-resistant bacteria represent a global public health problem. Refugees carrying these bacteria and living in inadequate shelters can spread these microorganisms. The aim of this study was to investigate the intestinal carriage of these bacteria in Syrian refugees in Lebanon. Between June and July 2019, 250 rectal swabs were collected from two refugee camps in North Lebanon. Swabs were cultured on different selective media. Antibiotic susceptibility testing was performed using the disk diffusion method. Carbapenemase-encoding genes and *mcr* genes were investigated using real-time polymerase chain reaction (RT-PCR) and standard polymerase chain reaction (PCR). Epidemiological relatedness was studied using multilocus sequence typing (MLST). From 250 rectal swabs, 16 carbapenem-resistant, 5 colistin-resistant, and 4 colistin and carbapenem-resistant *Enterobacteriaceae* were isolated. The isolates exhibited multidrug-resistant phenotypes. Seven *Klebsiella pneumoniae* isolates harboured the *bla*_{OXA-48} gene, and in addition four *K. pneumoniae* had mutations in the two component systems *pmrA*/*pmrB*, *phoP*/*phoQ* and co-harboured the *bla*_{NDM-1} gene. Moreover, the *bla*_{NDM-1} gene was detected in six *Escherichia coli* and three *Enterobacter cloacae* isolates. The remaining five *E. coli* isolates harboured the *mcr-1* gene. MLST results showed several sequence types, with a remarkable clonal dissemination. An urgent strategy needs to be adopted in order to avoid the spread of such resistance in highly crowded underserved communities.

Keywords: refugees; *Enterobacteriaceae*; NDM-1; *mcr-1*

1. Introduction

Antimicrobial resistance (AMR) has increased markedly in recent years. This is particularly important when facing complex infections in humans [1]. AMR is expected to result in severe mortality and economic losses that will increase the cycle of poverty [2]. This global public health concern causes huge clinical and economical losses, mainly in developing countries [3]. Thus, the Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) have recognised AMR as an urgent and global crisis and threat [4,5].

Carbapenems are a group of β -lactam drugs that are often used as last resort antibiotics to treat infections arising from multidrug-resistant (MDR) Gram-negative bacteria [6]. Unfortunately, carbapenemase-producing Gram negative bacteria have become a major concern around the world [7–9]. They are a growing concern because they confer resistance

to β -lactam antibiotics and other classes of antibiotics such as aminoglycosides, fluoroquinolones, and cotrimoxazole [10]. This reduces the possibility of treating infections due to such multi-resistant organisms [11].

Currently, carbapenemases in *Enterobacteriaceae* are found mainly in *Klebsiella pneumoniae*, *E. coli*, and other enterobacterial species, with a greater prevalence in southern Europe and Asia than in other parts of the world [6]. New Delhi metallo- β -lactamase-1 (NDM-1) is the most frequent carbapenemase found in Asia [12]. A recent study conducted in Nepal in 2019 [13] showed that the prevalence of MDR bacteria was 58% (199/343 isolates), with a prevalence of 12.5% for carbapenemase-producing bacteria and 3% for colistin-resistant bacteria. Interestingly, a study published in 2021 [14] in the same country, showed the rapid dissemination of carbapenem-resistant bacteria. From a total of 58 *K. pneumoniae* isolates, 60% (35/55) were carbapenem resistant with NDM-1 being the most dominant carbapenemase (80%).

On the other hand, *K. pneumoniae* carbapenemase (KPC) is mainly found in the United States and in Europe (France, Greece, and Italy) [12]. Several studies have reported an increased spread of KPC-producing *K. pneumoniae* such as the recent study conducted in Italy where 50% of carbapenem-resistant isolates were KPC-2 producers [15]. Moreover, OXA-48 carbapenemase is the main carbapenemase found in Turkey, Malta, and the Middle East [12]. Following its initial description in Turkey, OXA-48 carbapenemase has been reported in different parts of the world, mainly in the Mediterranean basin where several studies have described the emergence and spread of OXA-48 producing bacteria in that region [16].

Moreover, the emergence of MDR and extensively-drug-resistant (XDR) Gram-negative bacteria, as well as the lack of new agents against these pathogens, led to the reintroduction of colistin [17]. The use of colistin had been abandoned many years ago due to concerns about nephrotoxicity, however it is now regarded as a last resort option in infections caused by MDR bacteria [11,18–20]. The mechanisms of colistin resistance are complex and varied and can range from chromosomal to plasmidic. In addition, there is also a type of adaptive resistance that is simply related to the modification of lipopolysaccharides (LPS) moiety that changes the charge of the molecule, thus preventing binding [21–23].

The population of Lebanon was estimated to be 3,759,136 in 2007. This number increased to 4,822,000 in 2013, of which 21% were under 15 years of age and 12% over 60 years of age [24].

This dramatic increase is partly attributed to the influx of Syrian refugees following the declaration of war in Syria in 2012, which caused a large budget deficit exacerbated by the withdrawal of international organisations that had promised to participate in funding [25]. Many Syrian refugees currently in Lebanon live in poor living conditions with limited access to healthcare, clean water, and inadequate food [26,27]. There is no reliable health-related database to promote better care for this underserved population. More importantly, there is little data available concerning the epidemiology of antimicrobial resistance in the Syrian refugee population. The gastrointestinal tract is known to be a reservoir of Gram negative *Enterobacteriaceae*, and it has been shown that colonisation by MDR *Enterobacteriaceae* increase by at least two-fold the risk of subsequent infection [28]. Given that this may cause barriers to primary health care, we attempted to look at the prevalence of antimicrobial resistance, in particular carbapenem and colistin resistance genes which were isolated from bacteria, particularly *Enterobacteriaceae*, among Syrian refugees in North Lebanon, who live in unhygienic conditions, have limited toilet facilities, consume polluted water, very poor food quality, and live in overcrowded environments.

2. Materials and Methods

2.1. Study Design

Between June and July 2019, 250 rectal swabs were collected from the inhabitants of two Syrian refugee camps, located in North Lebanon, in the Akkar Governorate (Figure 1). One hundred random samples were collected from the Talhayat camp and another 150 from

the Bebnine camp. The swabs were grouped according to whether they came from residents of the same tent and/or family. The swabs were inserted through the rectal sphincter 1–1.5 inches (2–3 cm) and rotated gently. Tubes containing the swabs were placed in a refrigerator (2–8 °C), after checking that faecal material was visible on the tip of the swab. The swabs were directly shipped to the laboratory (less than 1.5 h away) where they were directly cultivated on Tryptic soy broth (TSB) medium (Becton Dickinson GmbH, Heidelberg, Germany).

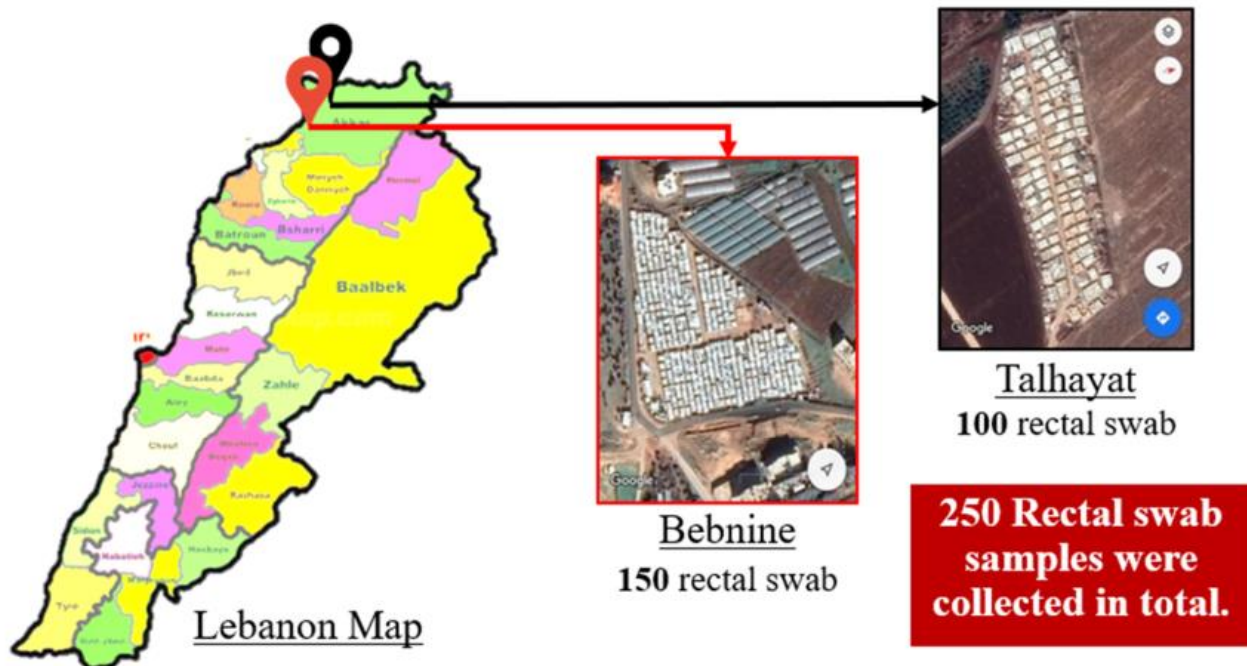


Figure 1. The location of the two camps in Lebanon where samples were taken.

2.2. Bacterial Identification

The rectal swabs were cultivated in the growth medium Tryptic soy broth (TSB) (Becton Dickinson GmbH, Heidelberg, Germany) and incubated for 24 h at 37 °C. Different selective media, including Lucie Bardet Jean-Marc Rolain (LBJMR) [29] and MacConkey agar (bioMérieux, Marcy l’Etoile, France) supplemented with Ertapenem (2 µg/mL) were then used to culture 100 µL of the enrichment medium. These media were used to screen colistin resistant and carbapenem resistant organisms, respectively. The selected isolates were then sent to the laboratory in Marseille, France. Bacterial identification at the species level was performed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) (Microflex; Bruker Daltonics).

2.3. Antibiotic Susceptibility Profile

The antibiotic susceptibility profile of the isolates was identified using the standard disk diffusion method on Mueller–Hinton agar (bioMérieux, Marcy l’Etoile, France). Sixteen different antibiotics were tested, including amoxicillin (20 µg), amoxicillin-clavulanic acid (20/10 µg), piperacillin-tazobactam (30/6 µg), cephalotin (30 µg), ceftriaxone (30 µg), cefepime (30 µg), ertapenem (10 µg), imipenem (10 µg), amikacin (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), Fosfomycin (200 µg), nitrofurantoin (100 µg), tobramycin (10 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), and colistin (10 µg) (bioMérieux, Marcy l’Etoile, France). The minimal inhibitory concentration (MIC) of colistin, ertapenem, and imipenem was identified using the microdilution and the E-test methods, respectively. Each strain was considered to be resistant to colistin, ertapenem, and imipenem if their

MICs were greater than 2 mg/L, 1 mg/L, and 8 mg/L, respectively. The results were interpreted according to the European Microbial Medical Sensitivity Committee (EUCAST) 2017 (http://www.Sfmicrobiology.Org/Userfiles/Files/Files/CASFM/CASF%20V2_0_MAI2017.PDF, accessed on 25 September 2020).

2.4. Phenotypic Detection of Carbapenemase Activity

Three phenotypic tests, the modified Hodge test (MHT), the modified Carba NP test (MCNP), and the ethylene diamine tetra-acetic acid (EDTA) test, were performed to detect the production of carbapenemase, as described previously [30,31].

2.5. DNA Extraction

The extraction of the bacterial DNA was performed by the EZ1-automatic robot (Qiagen Biorobot EZ1-, Tokyo, Japan) using an extraction kit (DNA EZ1, Qiagen, Hilden, Germany), following the manufacturer's instructions. The extracted DNA was kept at -20°C . In order to determine the genetic location of the carbapenemase and *mcr* genes, plasmid extraction was performed for the isolates that showed a positive PCR result for these genes using the GeneJET Plasmid Miniprep Kit (Thermo Fisher Scientific, Waltham, MA, USA).

2.6. Molecular Identification of Carbapenem and Colistin Resistance Genes

The presence of carbapenemase encoding genes such as *bla*_{OXA-48}, *bla*_{OXA-58}, *bla*_{KPC}, *bla*_{NDM}, and *bla*_{VIM} was investigated by the real-time PCR assay (RT-PCR) (Bio-Rad, CFX96 Touch™, Hercules, CA, USA) using specific primers, as previously described [32,33]. Moreover, RT-PCR for colistin resistant isolates was performed to detect the presence of *mcr-1*, *mcr-2*, *mcr-3*, *mcr-4*, *mcr-5*, and *mcr-8* genes using previously described primers and probes [34,35].

Standard PCR and sequencing of antibiotic resistance genes was performed on an automated ABI 3130 sequencer (PE Applied Biosystems, Foster City, CA, USA) using BigDye terminator chemistry. The sequenced genes were analysed using BlastN and BlastP and then compared with the ARG-ANNOT database.

All *mcr*-negative colistin-resistant bacteria were investigated for the presence of possible mutations in colistin-resistance genes such as *pmrA*, *pmrB*, *phoP*, *phoQ*, and *mgrB* by amplification and sequencing [32,36,37]. Those genes were compared with reference strains such as *E. coli* K-12 MG 1655 (NCBI GenBank accession no. U00096.3), *K. pneumoniae* MGH 78578 (NCBI GenBank accession no. CP000647), and *E. cloacae* ATCC 13047 (NCBI GenBank accession no. CP001918.1) using nps alignment software (<https://prabi.ibcp.fr/htm/site/web/home>, accessed on 18 February 2021).

In order to determine whether the recognised amino acid substitution resulting from a missense mutation could affect the function of the protein, PROVEAN (Protein Variation Effect Analyser) software (http://provean.jcvi.org/seq_submit.php, accessed on 18 February 2021) was used. If the score of the variant proteins was below or equal to a predefined threshold (-2.5), it was considered to have a “deleterious” effect, and if the score was above the threshold, it was predicted to have a “neutral” effect.

2.7. Multilocus Sequence Typing (MLST)

In order to determine the clonal relationship of the isolates, the MLST method was performed as described on the Institute Pasteur's MLST website (www.pasteur.fr/mlst, accessed on 23 March 2021).

3. Results

From 250 rectal swabs, 25 carbapenem and/or colistin resistant strains were isolated (10%). Among the isolates, 16 carbapenem-resistant strains were identified, namely, seven *Klebsiella pneumoniae*, six *Escherichia coli*, and three *Enterobacter cloacae*. Moreover, five *E. coli* isolates were resistant to colistin. The remaining four isolates were identified as

K. pneumoniae and were resistant to carbapenem and colistin. The antibiotic resistance profile is presented in Table 1 for carbapenem-resistant (CarbaR) strains and in Table 2 for colistin-resistant (ColiR) strains. Imipenem MICs ranged from 2 to >32 mg/L (Tables 1 and 2) and ertapenem MICs ranged from 4 to >32 mg/L (Tables 1 and 2). In contrast, according to the Unitary Minimum Inhibitory Concentration (UMIC) test, colistin MICs for *K. pneumoniae* and *E. coli* isolates were >64 mg/L and 4 mg/L, respectively (Table 2). The MCNP test and the MHT were positive for all carbapenem-resistant isolates, whereas the EDTA test was positive for all carbapenem-resistant isolates except for KP-2, KP-3, KP-4, and KP-5 isolates. Real-time PCR and standard PCR results showed that *E. coli* CarbaR (EC-1, EC-2, EC-3, EC-4, EC-5, and EC-6), *E. cloacae* CarbaR (Eclo-1, Eclo-2, and Eclo-3), and *K. pneumoniae* ColiR (KP-1, KP-2, KP-3, and KP-4) were positive for *bla*_{NDM} gene. Sequence analysis of detected carbapenemases identified the *bla*_{NDM-1} gene in positive strains (Tables 1 and 2). In contrast, results showed that *K. pneumoniae* CarbaR (KP-2, KP-3, KP-4, KP-5, KP-6, KP-7, and KP-8) were positive for the *bla*_{OXA} gene. Sequence analysis of detected carbapenemases identified the *bla*_{OXA-48} gene in positive strains (Table 1). Moreover, results showed that *K. pneumoniae* CarbaR strains (KP-6, KP-7, and KP-8) harboured, in addition, the *bla*_{NDM} gene. Sequence analysis of detected carbapenemases identified the *bla*_{NDM-1} gene in positive strains (Table 1). In addition, results showed that *E. coli* ColiR (EC-7, EC-8, EC-9, EC-10, and EC-11) were positive for the *mcr-1* gene and were identified as the *mcr-1* gene by sequencing (Table 2).

Table 1. Phenotypic and genotypic features of the carbapenem-resistant *Enterobacteriaceae* isolates.

| | Strain Name | Source | Antibiotic Susceptibility Profile | MAR Index | IMP MIC (µg/mL) | ERT MIC (µg/mL) | Carba NP Test | <i>bla</i> _{OXA-48} | <i>bla</i> _{NDM-1} | ST | Accession Number |
|---------------------------------------|---------------------------|--------|-----------------------------------|-----------|-----------------|-----------------|---------------|------------------------------|-----------------------------|------|----------------------|
| Same tent (same family) | <i>E. coli</i> EC-1 | BN | FF, F, AK, CS, CN | 0.68 | 12 | >32 | + | – | + | 361 | OL542491 |
| | <i>E. coli</i> EC-2 | BN | FF, F, AK, CS, CN | 0.68 | >32 | >32 | + | – | + | 361 | OL542492 |
| | <i>E. coli</i> EC-3 | BN | FF, F, AK, CS, CN | 0.68 | 16 | >32 | + | – | + | 361 | OL542493 |
| | <i>E. coli</i> EC-4 | BN | FF, F, AK, CS, CN | 0.68 | >32 | >32 | + | – | + | 1294 | OL542494 |
| | <i>E. coli</i> EC-5 | BN | FF, F, AK, CS, CN | 0.68 | >32 | >32 | + | – | + | 1294 | OL542495 |
| | <i>E. coli</i> EC-6 | TL | FF, F, AK, CS, CN | 0.68 | 4 | >32 | + | – | + | 648 | OL542496 |
| Same tent (same family) | <i>E. cloacae</i> Eclo-1 | BN | DO, CS | 0.87 | 4 | 12 | + | – | + | 182 | OL474360 |
| | <i>E. cloacae</i> Eclo-2 | BN | CS | 0.93 | 4 | 8 | + | – | + | 182 | OL474361 |
| | <i>E. cloacae</i> Eclo-3 | TL | CS | 0.93 | 4 | 8 | + | – | + | 1120 | OL474362 |
| Tents are close (Not the same family) | <i>K. pneumoniae</i> KP-2 | BN | AK, CS, CN | 0.81 | >32 | >32 | + | + | – | 16 | OL542509 |
| | <i>K. pneumoniae</i> KP-3 | BN | AK, CS, CN | 0.81 | >32 | >32 | + | + | – | 16 | OL542510 |
| | <i>K. pneumoniae</i> KP-4 | BN | TZP, CS | 0.87 | >32 | >32 | + | + | – | 16 | OL542511 |
| | <i>K. pneumoniae</i> KP-5 | BN | AK, CS, CN | 0.81 | >32 | >32 | + | + | – | 16 | OL542512 |
| Tents are close (Not the same family) | <i>K. pneumoniae</i> KP-6 | TL | DO, CS | 0.87 | >32 | >32 | + | + | + | 14 | OL542513 OL542506 |
| | <i>K. pneumoniae</i> KP-7 | TL | AK, DO, CS | 0.81 | >32 | >32 | + | + | + | 14 | OL542514 OL542507 |
| | <i>K. pneumoniae</i> KP-8 | TL | FF, AK, DO, CS | 0.75 | >32 | >32 | + | + | + | 14 | OL542515 OL542508 |

Fosfomycin (FF), nitrofurantoin (F), amikacin (AK), doxycycline (DO), colistin (CS), piperacillin-tazobactam (TZP), cefalotin (CF), gentamicin (CN), ertapenem (ERT), imipenem (IMP), minimum inhibitory concentration (MIC), sequence type (ST), multiple antibiotic resistance (MAR), Talhayat (TL), and Bebnine (BN).

Table 2. Phenotypic and genotypic features of the colistin and carbapenem-resistant *Enterobacteriaceae* isolates.

| | Strain Name | Source | Antibiotic Susceptibility Profile | MAR Index | IMP MIC (µg/mL) | ERT MIC (µg/mL) | UMIC Test (µg/mL) | Carba NP Test | <i>bla</i> _{NDM-1} | <i>mcr-1</i> | Mutations of the Associated Colistin-Resistance Proteins | ST | Accession Number |
|---------------------------------------|---------------------------------|--------|---|-----------|-----------------|-----------------|-------------------|---------------|-----------------------------|--------------|--|------|------------------|
| Tents are close (Not the same family) | <i>E. coli</i> EC-7 | BN | FEP, TZP, CRO, ERT, IMP, FF, F, AK, CN | 0.43 | – | – | 4 | – | – | + | – | 2001 | OL542497 |
| | <i>E. coli</i> EC-8 | BN | FEP, TPZ, CRO, ERT, IMP, FF, F, AK, CN, SXT | 0.37 | – | – | 4 | – | – | + | – | 2001 | OL542498 |
| | <i>E. coli</i> EC-9 | BN | FEP, TPZ, CRO, ERT, IMP, FF, F, AK, CN, SXT | 0.37 | – | – | 4 | – | – | + | – | 2001 | OL542499 |
| | <i>E. coli</i> EC-10 | BN | FEP, TPZ, CRO, ERT, IMP, FF, F, AK, CN | 0.43 | – | – | 4 | – | – | + | – | 101 | OL542500 |
| | <i>E. coli</i> EC-11 | TL | FEP, TPZ, CRO, ERT, IMP, FF, F, AK, CN, SXT | 0.37 | – | – | 4 | – | – | + | – | 4187 | OL542501 |
| Different tents (Same family) | <i>K. pneumoniae</i> ColiR KP-1 | BN | – | 1 | 4 | 4 | >64 | + | + | – | + | 944 | OL542502 |
| | <i>K. pneumoniae</i> ColiR KP-2 | BN | – | 1 | 2 | 4 | >64 | + | + | – | + | 944 | OL542503 |
| | <i>K. pneumoniae</i> ColiR KP-3 | BN | – | 1 | 2 | 4 | >64 | + | + | – | + | 944 | OL542504 |
| | <i>K. pneumoniae</i> ColiR KP-4 | BN | – | 1 | 2 | 4 | >64 | + | + | – | + | 944 | OL542505 |

Cefepime (FEP), piperacillin-tazobactam (TZP), ceftriaxone (CRO), ertapenem (ERT), imipenem (IMP), fosfomycin (FF), nitrofurantoin (F), amikacin (AK), gentamicin (CN), trimethoprim-sulfamethoxazole (SXT), doxycycline (DO), colistin (CS), minimum inhibitory concentration (MIC), sequence type (ST), multiple antibiotic resistance (MAR), Talhayat (TL), and Bebnine (BN).

Moreover, PCRs performed on extracted plasmids (from isolates that had a positive PCR for carbapenemase and *mcr-1* genes), showed a positive result for these genes assuming a plasmidic localisation.

Due to the absence of *mcr* genes in *K. pneumoniae* ColiR (Table 2), genes implicated in colistin resistance (*mgrB*, *pmrA*, *pmrB*, *phoP*, and *phoQ*) were amplified and sequenced. As shown in Table 3, the result of the sequence analysis showed that there was no mutation in the *mgrB*, *pmrA*, and *phoP* genes for the four colistin-resistant *K. pneumoniae* isolates. These isolates were resistant to colistin due to mutations in the *pmrB* and *phoQ* genes (Table 3). For the colistin resistant Kp-1 isolate, the analysis revealed a nucleotide deletion (C577del) in the *pmrB* gene, leading to a frameshift mutation and resulting in a defective protein. For the colistin resistant Kp-2, the analysis revealed a nucleotide insertion (G432_C433insG) in the *pmrB* gene, causing a frameshift mutation and resulting in a defective protein. For the Kp-3 isolate, the analysis also revealed a nucleotide insertion at position (T459_G460insC) in the *phoQ* gene leading to a frameshift mutation and resulting in a defective protein. Finally, for the Kp-4 isolate, the analysis showed two genetic modifications: a nucleotide insertion (G623_C624insG) in the *pmrB* gene leading to a frameshift mutation resulting in a defective protein, and a nucleotide insertion at position (T435_A436insT) in the *phoQ* gene causing a frameshift mutation resulting in a defective protein.

Table 3. Results of the nucleotide mutations of the colistin-resistant strains.

| Strain Name | Mgrb | PmrA | PmrB | PhoP | PhoQ | Accession Number |
|-------------|-------------|-------------|---------------|-------------|---------------|----------------------|
| Coli R Kp 1 | No mutation | No mutation | C577del | No mutation | No mutation | OL587685 |
| Coli R Kp 2 | No mutation | No mutation | G432_C433insG | No mutation | No mutation | OL587686 |
| Coli R Kp 3 | No mutation | No mutation | No mutation | No mutation | T459_G460insC | OL587683 |
| Coli R Kp 4 | No mutation | No mutation | G623_C624insG | No mutation | T435_A436insT | OL587687 OL587684 |

MLST analysis revealed that nine *E. coli* isolates belonged to six different sequence types (STs) including ST361 (*E. coli* CarbaR: EC-1, EC-2, EC-3), ST1294 (*E. coli* CarbaR: EC-4, EC-5), ST648 (*E. coli* CarbaR: EC-6) (Table 1), ST2001 (*E. coli* ColiR: EC-7, EC-8, EC-9), ST101 (*E. coli* ColiR: EC-10), and ST4187 (*E. coli* ColiR: EC-11). *K. pneumoniae* isolates had several STs such as ST14 (*K. pneumoniae* CarbaR: KP-2, KP-3, KP-4, KP-5), ST16 (*K. pneumoniae* CarbaR: KP-6, KP-7, KP-8) (Table 1), and ST944 (KP-1, KP-2, KP-3, KP-4) (Table 2), and the tree *E. cloacae* belonged to ST182 (Eclo-1, Eclo-2) and ST1120 (Eclo-3) (Table 1).

4. Discussion and Conclusions

MDR bacteria are now considered as a serious public health problem due to the limited range of antibiotics used to treat infections caused by these bacteria. The antimicrobial resistance crisis is mainly due to the misuse of these antibiotics in several sectors (human and animal) and the lack of discovery of new drugs [38]. Refugees coming from countries with a high occurrence of antimicrobial resistance can introduce the resistant isolates to their new country of residence. These refugees can spread these resistant bacteria, especially if they are living in shelters with poor living conditions. This study describes the intestinal carriage of carbapenem- and colistin-resistant bacteria particularly in *Enterobacteriaceae* among healthy Syrian refugees living in two shelters (Bebnine and Talhayat) in North Lebanon.

In this study, 25 carbapenem- and/or colistin-resistant isolates were collected from 250 rectal swabs isolated from healthy Syrian refugees. All isolates were resistant to the majority of antibiotics tested. PCR and sequencing showed the presence of 16 carbapenem-resistant isolates (6 *E. coli*, 7 *K. pneumoniae*, and 3 *E. cloacae*), 5 colistin-resistant isolates (*E. coli*), and 4 carbapenem and colistin-resistant isolates (*K. pneumoniae*) (Tables 1 and 2).

All carbapenem-resistant *E. coli* isolates (six isolates) harboured the *bla*_{NDM-1} gene. Five of them (EC-1, EC-2, EC-3, EC-4, and EC-5) were collected from the same shelter (Bebnine), and from the same tent; three isolates (EC-1, EC-2, and EC-3) belonged to ST361 and two (EC-4 and EC-5) belonged to ST1294. The *bla*_{NDM-1} *E. coli* ST361 had already

been reported in hospitalised patients in South Korea [39]; however, ST1294 is mainly found in animals and in the environment [40–45]. These results shed light on the clonal dissemination of these isolates between refugees, since the same clones were found in more than one person in the same tent, but also to the probable zoonotic or environmental origin of the ST1294 isolates. The sixth isolate (EC-6) belonged to ST648 and was collected from the Talhayat shelter. This clone was previously reported in different studies in hospitalised patients [46–48]. In Lebanon, NDM-1 producing *E. coli* isolates were first described in 2012 in Iraqi patients referred to Lebanon [49]. Moreover, NDM-1 producing *E. coli* was also reported in 2013 at a tertiary care centre in Lebanon [50]. To the best of our knowledge, this is the first report of intestinal carriage of NDM-1 producing *E. coli* in healthy individuals.

All carbapenem-resistant *K. pneumoniae* isolates (seven isolates: KP-2, KP-3, KP-4, KP-5, KP-6, KP-7, and KP-8) harboured the *bla*_{OXA-48} gene, of which three also had the *bla*_{NDM-1} gene (KP-6, KP-7, and KP-8). The carbapenem-resistant *K. pneumoniae* isolates (KP-2, KP-3, KP-4, and KP-5) harbouring the *OXA-48* gene had the same ST (ST16) and were collected from the same shelter (Bebnine) and from closely related tents. *OXA-48* producing *K. pneumoniae* ST16 was previously described in clinical isolates in Spain [51]. In Lebanon, several studies have reported the occurrence of *OXA-48* producing bacteria and *OXA-48* producing *K. pneumoniae*, all from hospital settings [49,52–54]. In addition, the remaining three isolates (KP-6, KP-7, and KP-8) harbouring both the *NDM-1* and the *OXA-48* genes, also had the same ST (ST14), and were collected from the Talhayat shelter from closely related tents. *NDM-1* and/or *OXA-48* producing *K. pneumoniae* ST14 have been reported worldwide [55–58], highlighting the rapid spread of this clone. In Lebanon, several studies have described *NDM-1* and/or *OXA-48* producing *K. pneumoniae* isolates [49,52,53]; however, this is the first description of the co-occurrence of *NDM-1* and *OXA-48* genes in the same isolate, which highlights the dissemination of plasmids carrying these resistance genes between isolates. Furthermore, results showed that *K. pneumoniae* isolates recovered from closely related tents in the same shelter belonged to the same clone. Syrian refugees in Lebanon live in inadequate shelters living in crowded conditions with poor sanitary facilities, which increases the spread of resistant bacteria. This explains the dissemination of the same clone in refugees living in closely related tents within the same shelter.

All carbapenem-resistant *E. cloacae* isolates (Eclo-1, Eclo-2, Eclo-3) harboured the *bla*_{NDM-1} gene, of which two (Eclo-1 and Eclo-2) were isolated from the same tent at the Bebnine shelter and belonged to the same clone (ST182). The remaining isolate belonged to ST1120 and was isolated from the Talhayat shelter. *NDM-1* producing *E. cloacae* ST182 have been reported in many countries, including China, Czech Republic, and Kenya [59,60]. This clone is of high concern because of its ability to disseminate rapidly, causing outbreaks as was the case in Mexico [61]. Hence, controls should be performed to avoid the rapid dissemination of such clones. To the best of our knowledge, this is the first detection of *NDM-1* producing *E. cloacae* in Lebanon.

In total, nine colistin-resistant isolates were collected, five *E. coli* (EC-7, EC-8, EC-9, EC-10, and EC-11) and four *K. pneumoniae* (ColiR KP-1, ColiR KP-2, ColiR KP-3, and ColiR KP-4).

All colistin-resistant *E. coli* isolates harboured the *mcr-1* gene, of which three (EC-7, EC-8, EC-9) belonged to the same clone ST2001 and were isolated from closely related tents at the Bebnine shelter. The remaining *E. coli* isolates belonged to ST101 (Bebnine shelter) and ST4187 (Talhayat shelter). *E. coli* ST2001 has been previously described in fecal samples from wild animals [62] and in an organic broiler farm [63]. ST4187 has been described in broiler chickens in Tunisia [64], thus pointing towards a zoonotic origin of our isolates, since the refugees are in close contact with wild animals. The ST101 clone has been reported in clinical samples from Brazil and Iran [65,66]. In Lebanon, *E. coli* harbouring the *mcr-1* gene has been described in few studies in hospitalised patients [67], however, no other study has highlighted its presence in healthy individuals. To the best of our knowledge, this is the first description in Lebanon of the intestinal carriage of

E. coli isolates harbouring the *mcr-1* gene in healthy individuals. This is alarming, since the plasmid carrying the *mcr-1* gene could disseminate between species, making them resistant to one of our last resort antibiotics.

All colistin-resistant *K. pneumoniae* isolates harboured the *bla*_{NDM-1} gene and had mutations in the *PmrB* and/or *PhoQ* genes (Table 3). They were all isolated from the same family (in the Bebnine camp) and belonged to the same clone ST944. An ST944 clone harbouring the *mcr-1* gene has been previously described in chicken meat from western Algeria [68]. A recent study conducted in Egypt [69] described colistin and carbapenem resistance in *K. pneumoniae* strains isolated from chicken and humans. The study showed that all *K. pneumoniae* were MDR, with 45.9% harbouring a β -lactamase gene with a carbapenemase gene, 18.9% harbouring a β -lactamase gene with the *mcr-1* gene, and 13.5% harbouring a β -lactamase gene with both carbapenemase and *mcr-1* genes. In Lebanon, few studies have described colistin-resistance in *K. pneumoniae* isolates such as those in 2015 [70] and 2017 [71]. Our study showed new mutations (Table 3) in the *pmrB* and/or *phoQ* genes resulting in a defective protein with a stop codon. Interestingly, a study conducted in Lebanon in 2018 [72] described colistin-resistance in clinical *K. pneumoniae* isolates due to mutations in the *mgrB*, *pmrB*, and *phoQ* genes. These isolates also harboured the *bla*_{NDM-5} gene. Our study reported for the first time in Lebanon, colistin-resistance *K. pneumoniae* isolates with new mutations in *pmrB* and/or *phoQ* genes and harbouring the *bla*_{NDM-1} gene.

In conclusion, the intestinal carriage of MDR bacteria in community settings is of great concern. Our study reported for the first time the intestinal carriage of carbapenem- and/or colistin-resistant isolates in refugees, which is alarming since plasmids carrying resistance genes can spread between bacteria and refugees within the same shelter. Syrian refugees come from a country with a high prevalence of antimicrobial resistance where antibiotics can be taken without prescription, thus they can spread MDR bacteria to their final destination, in this case, Lebanon. In addition, these refugees are living in inadequate shelters which can contribute to the spread of these bacteria. Molecular and epidemiological studies are essential in order to better understand the mode of transmission of these microorganisms. An urgent strategy must therefore be adopted to limit the spread of these MDR bacteria within the community.

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THESE DE DOCTORAT

Faraj FAJLOUN

Titre : Maladies tropicales négligées à manifestations cutanées : vers un développement de nouvelles stratégies préventives, de diagnostic et de traitement.

Mots clés : Maladies tropicales négligées à manifestations cutanées, Bénin, Ulcère de Buruli, Lèpre, dépistage intégré

Résumé : Les maladies tropicales négligées à manifestations cutanées (MTN-C) sont un problème majeur de santé pour les populations à risque. Le Bénin, endémique pour certaines de ces maladies, a mis en place un système de lutte spécifique formé de programmes nationaux et de structures de diagnostic et de prise en charge des patients. L'objectif de ce travail de thèse a été de valoriser les efforts importants réalisés au fil des années par les équipes du Centre de Dépistage et de Traitement de la Lèpre et de l'Ulcère de Buruli (CDTLUB) de Pobè au sud du Bénin, pour faciliter la prise en charge de l'ulcère de Buruli (UB) et d'autres MTN-C. Dans un premier temps, nous avons analysé les résultats de 10 ans de diagnostic de l'UB par le laboratoire du CDTLUB de Pobè. Ce travail a particulièrement montré le rôle majeur du laboratoire comme laboratoire expert en biologie moléculaire pour le diagnostic des MTN-C. Dans un deuxième temps, nous avons montré l'importance des consultations avec des équipes mobiles sur le terrain, pour la prise en charge

intégrée des MTN-C. Ces travaux ont notamment mis en évidence des maladies sous-déclarées dans des villages reculés et des épidémies qui nécessitent des interventions rapides pour leur contrôle. Ils ont également montré l'efficacité de ces consultations foraines dans la réduction du fardeau des maladies à manifestations cutanées. Enfin, nous avons participé à la mise en place d'un test de détection moléculaire de la résistance aux antiléproux au CDTLUB de Pobè. Ce travail fait suite à une demande des médecins du centre pour la prise en charge adaptée des patients lépreux. L'organisation mise en place par le CDTLUB de Pobè pour la prise en charge des MTN-C constitue un exemple pionnier qu'il est important de mettre en lumière afin que d'autres pays endémiques à ces maladies puissent bénéficier de cette expérience pour développer des structures efficaces permettant de diminuer le fardeau des MTN-C.

Title: Neglected tropical diseases with cutaneous manifestations: towards the development of new preventive, diagnostic and treatment strategies.

Keywords: Neglected tropical diseases with cutaneous manifestations, Benin, Buruli ulcer, Leprosy, Integrated screening

Abstract: Neglected tropical diseases with cutaneous manifestations (NTD-C) are a major health problem for populations at risk. Benin, endemic for some of these diseases, has set up a specific control system made up by national programs and structures for diagnosis and patient care. The objective of this thesis work was to highlight the significant efforts made over the years by the teams of the Center of Screening and Treatment of Leprosy and Buruli Ulcer (CDTLUB) in Pobè in southern Benin, to facilitate the management of Buruli ulcer (BU) and other NTD-C. First, we analyzed the results of 10 years of BU diagnosis by the CDTLUB laboratory in Pobè. This work has particularly shown the major role of the laboratory as an expert in molecular biology for NTD-C diagnosis. Secondly, we showed the importance of consultations with mobile teams in the integrated

management of NTD-C in the field. This work has highlighted under-reported diseases in remote villages and epidemics that require rapid interventions for control. They also showed the effectiveness of these mobile consultations in reducing the burden of this diseases with cutaneous manifestations. Finally, we participated in the implementation of a molecular detection test for leprosy drugs resistance at the CDTLUB of Pobè. This work follows a request from the doctors of the center for the appropriate care of leprosy patients.

The organization set up by the CDTLUB of Pobè for the management of NTD-C constitutes a pioneering example that should be valorised so that other countries endemic to these diseases can benefit from this experience to develop effective structures to reduce the burden of NTD-C.