Increscent journey of anti-leprosy drug development: A review

Sakshi Gautam¹², Devesh Sharma ¹, Sakshi Singh¹, Nirmala Deo¹, Anjana Goel², Vivek Kumar Gupta¹ and Deepa Bisht¹*

¹ Department of Biochemistry, ICMR-National JALMA Institute for Leprosy and Other Mycobacterial Diseases, Tajganj, Agra 282001, India.
² Department of Biotechnology, GLA University, NH-2, Mathura-Delhi Road, Mathura 281406, India.

*Correspondence:
Deepa Bisht: abd1109@rediffmail.com

Abstract

Leprosy, a chronic granulomatous disease generally caused by Mycobacterium leprae and Mycobacterium lepromatosis, still exists as a serious public health concern particularly in developing countries. With the introduction of Multi-Drug Therapy (MDT) by the World Health Organization (WHO) since 1980, the prevalence of leprosy has declined globally. In the past, acid-fast bacilli frequently developed resistance to both first-line (dapsone, rifampicin, and clofazimine) and second-line drugs (fluoroquinolones, minocycline, and clarithromycin). According to previous research, it is reported that genes like rpoB, gyrA, and folP play a role in drug resistance. Considering its exceptionally modest pace of growth, it is difficult to cultivate M. leprae in a laboratory environment on a synthetic medium. Thus, studies on animal models have assisted in the evaluation of anti-leprosy drugs and documentation of drug-resistant strains, as well as other basic immunological investigations examining the efficacy of vaccinations. In addition to the conventionally administered MDT treatments, several newly developed drugs have shown more impressive results, along with combinational therapies of moxifloxacin-based regimens, having much better efficacy. This review focuses on the increscent journey of anti-leprosy drugs to treat the disease and highlights the relevance of animal models in research and development of anti-leprosy drugs.

Keywords: Antibiotic, pharmacokinetics, drugs-mode of action, animal models, Mycobacterium leprae, vaccine

Introduction
The etiological agents of leprosy *M. leprae* and *M. lepromatosis*, the second causal agent of Hansen's disease, are still prevalent in several countries, making it an important public health concern. Skin lesions, damage to tissues, abnormalities, and a weakened immune system that leads to nerve damage are the prominent symptoms of the disease\(^1\). The disease has various clinical manifestations, with tuberculoid leprosy (TL) and lepromatous leprosy (LL) occupying opposing ends of the spectrum. Inability to cultivate *M. leprae* in vitro, has led to use of animal models to test novel medications, vaccines, and fundamental pathogenesis mechanisms\(^2\).

Incidence of leprosy has decreased worldwide since 1980s, with the introduction of MDT by the WHO. However, the global annual new case detection rate has remained almost constant over the last decade. This has been evidenced by the fact that *M. leprae* is still getting spread by untreated patients\(^3\). Previous research has examined mechanisms of resistance of leprosy to dapsone (*folP1*)\(^4\), rifampicin (*rpoB*)\(^5\), and ofloxacin (*gyrA*)\(^6\). However, only a small number of mice footpad experiments have shown clofazimine resistance\(^7\).

Nerve damage may occur before diagnosis, during treatment or even after, which should be detected and treated promptly to avoid deformity. The major reason for nerve injury and lifelong impairments are Lepra Reactions (LR). These can be either Type 1 leprosy reaction (T1LR) or Type 2 leprosy reaction (T2LR)\(^8\). Currently, there are no generally accepted laboratory markers for LR. It is necessary to develop more pharmacological and immunotherapeutic strategies to protect neurologic function as neuropathy still poses a challenge, particularly if diagnosis and treatment are deferred\(^9\).

Current findings by Yamaguchi and coworkers\(^10\) revealed that fluoroquinolones DC-159a and sitafloxacin are more effective than moxifloxacin against wild-type and mutant *M. leprae* DNA gyrase. Gautam et al.\(^11\) have recently reviewed the biomarkers for *M. leprae* diagnosis and the efficacy of immunization in reducing leprosy cases. A critical MDT approach is important in addition to an accurate disease diagnosis\(^11\).

**Leprosy classification**

Ridley and Jopling\(^12\) classified leprosy in 1966, based on immunological, pathological, and microbiological criteria\(^1\). In 1981, WHO categorized leprosy into two subtypes: Paucibacillary (PB) and Multibacillary (MB) based on the presence or absence of acid-fast bacilli with clinical symptoms\(^13\). The classification of leprosy according to the WHO and Ridley-Jopling systems is
depicted in Figure 1. Arif et al. comprehensively reviewed the classification of leprosy and suggested that it would be cost-effective and safe for patients if the correct classification strategies were used to ensure the effectiveness of the control program. 

Figure 1. A schematic representation of classification of leprosy given by WHO and Ridley & Jopling

Importance of animal models in anti-leprosy drugs development

Research on live animal models since the early 20th century, has aided in developing therapeutic drugs and assessing drug toxicity. Due to the anatomical and physiological similarities between humans and animals, particularly mammals, researchers have examined new therapies in animal models before utilizing them in humans. Animal models are used in leprosy research to evaluate anti-leprosy medications, cataloging of drug-resistant strains, and conduct basic immunological studies, including vaccine efficacy testing. Johnstone discussed the early attempts to develop *M. leprae* in diverse species including mammals, birds, and cold-blooded species. The expensive and logistically challenging mouse footpad assay (MFP) requires months of care of dozens or hundreds of mice. The
usual time for MFP studies to provide data on "culture and sensitivity" for \textit{M.leprae} is 12 months or more. It enabled researchers to assess the effectiveness of anti-\textit{M.leprae} medications before starting a clinical investigation and was still the best approach available 40 to 50 years ago\textsuperscript{16}.

Apart from humans, armadillos served as a model for \textit{M.leprae} infection in 1971\textsuperscript{17}. Many functional, physiological, and anatomical features of armadillo’s leprosy are comparable to those seen in human leprosy. Armadillos also exhibit the whole clinical spectrum of leprosy and severe peripheral nerve damage. This knowledge improvement has permitted testing of novel therapeutic and diagnostic regimens in armadillos that have provided new insights into the oldest known neurodegenerative disease\textsuperscript{18}. The liver, spleen, lymph nodes, lips, tongue, nose, nasal mucosa, skin, bone marrow, eyes, lungs, and nerves are among the organs where \textit{M.leprae}-infected macrophages have been shown to infiltrate the armadillo\textsuperscript{19}. Using the specific repeating element RLEP of DNA extracted from an armadillo's ear, liver, and lungs, Vera-cabrera \textit{et al.} established the presence of \textit{M.leprae} in tissues by PCR testing\textsuperscript{20}.

Recent research on mice and armadillos led to the discovery of LepVax, a specialized subunit vaccine that provides excellent pre-and post-exposure prophylaxis against \textit{M.leprae} infection. Mice vaccinated with LepVax-vaccine had bacterial loads that were about 85\% lower than those seen in animals 12 months later. A study found that when LepVax was given to armadillos that had been exposed to \textit{M.leprae}, it prevented and slowed down the damage to the motor and sensory nerves\textsuperscript{21}. Adams \textit{et al.}\textsuperscript{22} recently discussed \textit{M.leprae} susceptibility and drug resistance, focusing on \textit{M. leprae}-induced granuloma, its histopathology, cellular composition, immunological agents produced by the cells, and their ability to kill or, conversely, provide a niche for \textit{M.leprae}.

**Anti-leprosy drugs**

Efforts have been made to develop new treatment plans that can shorten treatment time and increase compliance while keeping or improving the therapeutic benefits of existing plans. Based on pathophysiological data, WHO made very useful medication blister packs. Numerous drugs and methods were used to treat leprosy, such as potassium iodide, arsenic, antimony, copper, vaccines, aniline dyes, mercury, gold, iodine, thymol, trychnine, sodium salicylate, carbolic acid, various kinds of baths, radium, electric current, X-ray, and surgical procedures such as nerve stretching, bleeding, and ulcer removal\textsuperscript{23}.
Before the development of antibiotics, chaulmoogra oil was the first drug used to treat leprosy in the early 20th century, and it was widely regarded as an effective leprosy therapy. The oil is extracted from the seeds of *Hydnocarpus wightianus* and was originally administered topically to leprous regions of the body or consumed internally24. The presence of cyclopentenyl fatty acids in seed oil was linked to its anti-leprotic properties25. When taken orally and intramuscularly, chaulmoogra oil had little effect and produced nausea and stomach discomfort. Therefore, patients used to refuse to take it. Also use of chaulmoogra oil deep injections was disliked as very painful. As a result, it was replaced with a sulfone medication24. In 1941, Promin was the first sulfone medication used to treat leprosy. Dr. Guy Faget of Carville, Louisiana, were the first to test it26.

(A) **FIRST-LINE DRUGS**

From 1982, WHO has recommended Clofazimine, Rifampicin, and Dapsone as the first-line medications for treating leprosy13. They are the cornerstone antibiotics of multi drug therapy (MDT).

(I) **Dapsone (DDS)**

(i) **Name of the compound :** 4, 4'-diaminodiphenylsulfone

The usage of dapsone was spurred by a side effect of promin. It possesses antimicrobial/antiprotozoal and anti-inflammatory properties27.

(ii) **Mode of action:**

It prevents dihydrofolic acid production by competing with para-aminobenzoic acid (PABA) for the active site of dihydropteroate synthase (DHPS). Dihydrofolic acid is a critical component of *M. leprae* in nucleic acid biosynthesis28.

(iii) **Clinical pharmacokinetics profile:**

Dapsone has an approximate bio-availability of 86% and is rapidly absorbed by the digestive tract. In severe leprosy, the absorption rate is impaired29. When it reaches the liver through enterohepatic circulation, it is metabolized by N-hydroxylation to produce lethal hydroxylamines or acetylation to produce innocuous acetyl-dapsone, with an elimination half-life of 24-30 hrs30,31. Hemolytic anemia and dapsone hypersensitivity syndrome (DHS) are the consequences that emerge from hydroxylamine (toxic metabolite of dapsone)32. Peak serum concentrations are attained in 2- 8 hrs33, and dosage recommendations are 1-2 mg/kg34. It is excreted unaltered in urine (conc. 20%), but after being conjugated with glucuronic acid is eliminated as water-soluble metabolites (conc. 70–85%)27.
(iv) Resistance to dapsone

Resistance to dapsone is caused due to mutation in codon 55 of the folP gene prompted by the substitution of leucine with proline\textsuperscript{35}. According to Nisha et al.\textsuperscript{36} CID21480113 (4-(2-fluorophenylsulfonyl) benzenamine) has the potential to be developed as a medication for dapsone-resistant leprosy patients\textsuperscript{36}.

(II) Rifampicin (RFP) or Rifampin

(i) Name of the Compound: 3-(4-methyl-1-piperazinyl)-imino-methylrifamycin

(ii) Mode of action:

Rifampin inhibits RNA synthesis by binding the β sub-unit of DNA-dependent RNA polymerase. Thus, no bacterial protein is synthesized, and \textit{M.leprae} do not replicate\textsuperscript{37}.

(iii) Clinical pharmacokinetics profile:

Rifampicin is almost fully absorbed from the digestive system when taken on an empty stomach. It mostly undergoes deacetylation in the hepatocytes. It is eliminated through urine (30\%) and faeces (60–65\%), and its half-life is approximately 2.5 hrs. Serum peak concentrations of 10 g/ml are observed between 1 and 2 hrs. A single dosage of 600 mg of rifampin kills 92.1\% of the total bacilli\textsuperscript{38}.

(iv) Resistance to rifampicin:

Rifampin resistance in \textit{M.leprae} is caused by missense mutation in the rpoB gene, which code for beta-subunit of the essential enzyme RNA polymerase. This was assessed by PCR amplification of a specific region of the rpoB gene, followed by single-strand conformational polymorphism analysis (PCR-SSCP)\textsuperscript{39}. Richardus and co-workers\textsuperscript{40} reported that single-dose rifampicin (SDR) for post-exposure prophylaxis is safe and interpreted that it can be implemented into various leprosy control programs.

(III) Clofazimine (CLF)

(i) Compound name: 3-(p-chloroanilino)-10-(p-chlorophenyl)-2,10]-dihydro (isopropylimino)-phenazine.

Clofazimine, initially known as B663, is a lipophilic riminophenazine antibiotic with antimycobacterial action and anti-inflammatory properties\textsuperscript{41}. An important feature of riminophenazine is
phenazine nucleus with an alkyl-imino and phenyl substituent, which is necessary for antibacterial activity\textsuperscript{42}. Accumulation of CLF crystals in the colon can lead to fatal and severe CLF-induced enteropathy and skin pigmentation\textsuperscript{43}.

\textit{(ii) Mode of action:}

CLF's mode of action has been the subject of several investigations. It binds to DNA primarily in G-C (guanine-cytosine) rich regions of mycobacterial DNA and inhibits DNA replication. Its lipophilicity may result in membrane disruption and dysfunction. Intracellular reactive oxygen species (ROS) like H\textsubscript{2}O\textsubscript{2} and super oxide, which have antibacterial characteristics, are generated by CLF via redox cycling. Later, it was discovered that the bactericidal efficacy of CLF is due to its interaction with the bacterial membrane phospholipids to generate antimicrobial lysophospholipids, which may result from the combined membrane destabilizing effects of both CLF and lysophospholipids, interfering with K\textsuperscript{+} uptake and eventually ATP production\textsuperscript{44}. Although CLF's anti-inflammatory effects are probably due to it suppressing the T lymphocyte activation and proliferation, it might also block the function of Kv1.3 potassium channel\textsuperscript{45}.

\textit{(iii) Clinical pharmacokinetics profile:}

Oral absorption of CLF is gradual and dose-dependent\textsuperscript{33}. According to Feng \textit{et al.} metabolite I is the result of a hydrolytic dehalogenation process, whereas metabolite II is the result of hydrolytic deamination followed by glucuronidation, and its half-life is varied, ranging from 10-70 days in single and multiple-dose studies\textsuperscript{46}. Its peak plasma concentration was 407.6 ng/g between 4 and 8 hrs. after a single 200 mg oral dosage when administered 10 min after breakfast. When dosage is raised, the drug's fecal excretion rises, and approximately 1\% of the dosage's metabolites are excreted in urine\textsuperscript{33}. Recently, Yaun \textit{et al.}\textsuperscript{47} suggested that CLF may play an important role in controlling future coronavirus outbreaks.

\textbf{(B) SECOND LINE DRUGS}

Second-line drugs are mainly fluoroquinolones, minocycline and clarithromycin.

\textit{(I) Fluoroquinolones}

Fluoroquinolones (FQs) pefloxacin, ofloxacin, norfloxacin, ciprofloxacin, and enoxacin are most well-investigated for their antibacterial activity against gram-negative and gram-positive microorganisms\textsuperscript{33}. For PB individual with single lesion, ofloxacin is recommended in current MDT regimens\textsuperscript{48}.
(i) **Mode of action:**

Fluoroquinolones mainly target two bacterial enzymes – gyrase and topoisomerase IV as ternary complexes on DNA and prevent replication forks and transcription complexes from progressing ahead, killing certain bacteria within hrs.\(^{49}\).

(ii) **Clinical pharmacokinetic profile:**

Absorption rate of ofloxacin (OFLO) is around 98%. It is mostly eliminated unaltered by the kidneys and its half-life is approximately 5-8 hrs., while pefloxacin is 10-12 hrs. After 2hrs., serum concentrations reach a peak of 2.9 g/ml\(^{50,51}\). Except for ofloxacin, all fluoroquinolones are metabolized by the liver\(^{51}\). Moxifloxacin has strong immunomodulatory characteristics, like suppression of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), which are implicated in the development of LR, particularly type 2, and contribute to LL patients’ homeostasis\(^{52}\).

(iii) **Resistance to fluoroquinolone:**

Employing PCR experiment, Raharolahy et al.\(^{53}\) demonstrated that the A91V (Ala→Val at position 91) mutation in the gyrA gene, which codes for the A sub-unit of DNA gyrase, is the major cause of quinolone resistance.

(II) **Minocycline (MINO)**

(i) **Name of the Compound**: 7-dimethylamino-6-dimethyl-6-deoxytetracycline

Minocycline, lipophilic in nature, is a tetracycline antibiotic with significant activity against *M. leprae*, which enables it to penetrate the bacterial cell wall\(^{54}\). According to Narang et al.\(^{55}\) neuritis improved when minocycline was administered to patients with type 2 lepra response. MINO is most effective against *M. leprae* when used in conjunction with DAP and RFP and clarithromycin\(^{56}\).

(ii) **Mode of action:**

Minocycline's mechanism of action against *M. leprae* is unknown, although it is presumed to be identical to that of all tetracyclines, which inhibit protein synthesis. Tetracyclines bind reversibly to the 30S sub-unit of the ribosome, preventing aminoacyl-tRNA from binding to the mRNA-ribosome complex and therefore inhibiting protein synthesis\(^{57}\). The molecular basis of minocycline resistance...
in *M. leprae* has not been investigated due to the absence of resistant mutants and also because minocycline has mostly been used with rifampin and ofloxacin to treat single-lesion PB leprosy.\(^{58}\)

(iii) Clinical pharmacokinetics profile

Absorption of minocycline in the jejunum ranges from 95-100% \(^{59}\), and it metabolizes in the liver. Mass spectral studies show that it is metabolized into 9-hydroxyminocycline and two other mono-N-demethylated derivatives\(^{60}\). Within 2 hours of a 0.2 g/ml administration, peak serum concentrations of 2-4 g/ml (mean 1.84 g/ml) were detected, and the half-life has been estimated to be between 6 and 11 hours. The recommended daily dose is 100 mg. It is mostly excreted in the feces and excreted at a modest rate (5–12%) in urine.\(^{59}\)

(III) Clarithromycin

(i) Name of the Compound: 6-O-methylerythromycin

Clarithromycin (CLZ) is a semisynthetic macrolide with bactericidal activity against Hansen’s bacilli. It differs from erythromycin by possessing a methyl substitution at 6\(^{\text{th}}\) position of macrolide ring.\(^{61}\) It has anti-inflammatory actions and modulatory effects on cytokines and chemokine production while it has immuno-modulatory effects on inflammatory cells, fibroblasts, and epithelial cells.\(^{62}\)

(ii) Mode of action:

Its mode of action against *M. leprae* is unclear, it is assumed to be comparable to macrolides, which inhibit protein synthesis by binding to the 50S sub-unit of the mycobacterial ribosome specifically targeting the 23S.\(^{54}\)

(iii) Clinical pharmacokinetics profile

Clarithromycin is readily absorbed from the gastrointestinal tract, although its systemic availability is decreased owing to first-pass metabolism (by roughly 55%). It degrades quickly and is transformed into active 14-hydroxy (R) metabolite, with a half-life of 6-7 hrs. The drug concentration peaks at 1 g/ml after 1-4 hrs and is mostly eliminated in urine with the parent component.\(^{51}\)

(iv) Resistance to clarithromycin:

Resistance to macrolides appears to be related to reduction in the drug binding to ribosomes and is associated with alterations or missense mutations in 23S rRNA inside the large ribosomal subunit.
For leprosy cases with rifampicin resistance or allergy, CLZ may be recommended as an alternative treatment.\textsuperscript{61,63}

### Significant side effects of first and second-line anti-leprosy drugs

During the treatment, patients experienced some common side effects after taking first-line and second-line drugs. Table 1 represents the side effects of drugs according to the incidence and severity.

#### Table 1: Anti-leprosy medicine’s side effects

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Severity</th>
<th>Incidence</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dapsone</td>
<td>Fever, hepatitis, skin reactions, headache</td>
<td>Very common (&gt; 80%)</td>
<td>\textsuperscript{64}</td>
</tr>
<tr>
<td></td>
<td>Lymphadenopathy, pruritus, leukocytosis, anemia, eosinophilia</td>
<td>Common (50–80%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mucosal involvement, exfoliative dermatitis splenomegaly, nausea and vomiting, atypical lymphocytosis, hemolytic anemia</td>
<td>Less common (10–50%)</td>
<td></td>
</tr>
<tr>
<td>Rifampicin</td>
<td>Cutaneous problem</td>
<td>Uncommon (≤5%)</td>
<td>\textsuperscript{65}</td>
</tr>
<tr>
<td></td>
<td>Gastrointestinal manifestations</td>
<td>Variable</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hepatitis</td>
<td>Common (≤1%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Thrombocytopenic purpura</td>
<td>Very uncommon</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hemolytic anemia, shortness of breath, renal failure</td>
<td>Rare</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Flu Syndrome: fever, chills, and sometimes headache, dizziness, and bone pain</td>
<td>Uncommon during the initial weeks</td>
<td></td>
</tr>
<tr>
<td>Clofazimine</td>
<td>Skin reactions</td>
<td>NA</td>
<td>\textsuperscript{66}</td>
</tr>
<tr>
<td></td>
<td>Gastrointestinal manifestation</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Eye toxicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td>Gastrointestinal symptoms</td>
<td>15.3%</td>
<td>\textsuperscript{67}</td>
</tr>
<tr>
<td></td>
<td>Cutaneous symptoms</td>
<td>20.3%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Musculoskeletal problems</td>
<td>6.8%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Central nervous system problems</td>
<td>11.9%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Peripheral nervous system problems</td>
<td>6.8%</td>
<td></td>
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<tr>
<td></td>
<td>Cardiovascular problem</td>
<td>18.6%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>20.3%</td>
<td></td>
</tr>
<tr>
<td>Minocycline</td>
<td>Headache</td>
<td>(up to 23%) Very Common</td>
<td>\textsuperscript{68}</td>
</tr>
<tr>
<td></td>
<td>Gastrointestinal symptoms</td>
<td>Common (1–10%)</td>
<td></td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>Gastrointestinal manifestations, Cutaneous problem</td>
<td>NA</td>
<td>\textsuperscript{69}</td>
</tr>
</tbody>
</table>

*Abbreviation: NA: not available

#### Multi-Drug Therapy (MDT)
Initially, MDT was prescribed for two years or until the smear of an MB case tested negative \(^{13,70}\). Six months course of rifampicin and dapsone, followed by rifampicin once a month was advised for PB case. Reducing the set durations of MB therapy from 24 months to 12 months in 1988 was the most significant modification made\(^{71}\). WHO also recommended a single-dose regimen for individuals with just one PB lesion\(^{72}\). Despite this development, new-case detection rates are steady in nations like Brazil and India, with highest endemic leprosy prevalence. This indicates that using antibiotics alone is ineffective in controlling the illness. Table 2 shows the detailed profile of drug therapies.

According to Anusuya and Natarajan \(^{73}\), the novel multi-targeted therapy for leprosy aims to reduce drug resistance and increase therapeutic efficacy. The goal of multi-targeted therapy is to prevent drug resistance by focusing on several significant enzymes in the bacterial metabolic pathway (Mur C, D, E and F). Conserved active sites of these enzymes were selected for multi-targeted therapy. Overview of the whole process of drug discovery has been represented in Figure 2. There are three main stages: the infection stage, when disease spreads to a healthy person, the observation stage, when main symptoms develop and are observed; and the experimental stage, when animals and then the person affected with leprosy are subjected to treatment. After the investigations and validation of a particular drug, WHO grants its approval.

MDT regimens for the treatment of leprosy have changed significantly, particularly in terms of treatment durations. The potential benefits of such a modification include simplification of the treatment regimen, shortened time period for MB cases, and reduced impact of mis-classification of leprosy cases. According to Rao et al.,\(^{74}\) uniform MDT (U-MDT) for six months was well accepted and appeared to have minimal therapeutic impact on PB leprosy, but it was too brief a regimen to adequately treat MB leprosy. WHO\(^{75}\) recommended accompanied-MDT (AMDT) to aid populations who live in remote border regions, urban slums, areas of civil unrest, as well as migrant workers. Initially, MB cases had fixed-duration therapy (FDT) for 24 months; later it was reduced to 12 months, whereas PB cases received treatment for 6 months\(^{76}\).

In the treatment of leprosy, other drugs with distinct modes of action have been introduced. These drugs inhibit various molecular processes like replication, transcription, and translation. In fluoroquinolone family, moxifloxacin (MXFX), sparfloxacin (SPFX), and levofloxacin (LVFX) are bactericidal antibiotics. LVFX inhibits bacterial DNA synthesis, SPFX inhibits topoisomerase II (DNA gyrase) and topoisomerase, and MXFX inhibits the replication-required DNA gyrase\(^{54}\).
Ansamycins rifabutin (LM 427), rifapentine (DL 473), and R-76-1 (isobutylpiperazinyl rifampicin SV) inhibit the DNA-dependent RNA polymerase of bacteria. Fusidic acid inhibits the translocation factor G during protein synthesis\(^5\). Beta-lactam antibiotics cephaloridine, cefuroxime, and amoxicillin plus clavulanic acid inhibit the formation of cell wall peptidoglycan layer. Bedaquiline or diarylquinoline (TMC207 or R207910) blocks the proton pump of mycobacterial adenosine 5'-triphosphate synthase (108) and nitazoxanide (NTZ) inhibits respiration completely in *M. leprae*\(^7\).
### Table 2: Detailed profile of drug therapies including (i) Multi-Drug Therapy, (ii) Combination of newer anti-leprosy drugs, (iii) Moxifloxacin-based regimens

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Year</th>
<th>Therapy</th>
<th>Drug Recommendation</th>
<th>No. of subjects that completed treatment/ Total no. of subjects</th>
<th>Statistical analysis</th>
<th>Region of study</th>
<th>Outcomes</th>
<th>Remarks</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2004</td>
<td>Accompanied multi-drug therapy (A-MDT)</td>
<td>NA</td>
<td>962/1000</td>
<td>NA</td>
<td>Madagascar</td>
<td>It was advised for the treatment of leprosy-affected patients who were unable to visit monthly or flexible RFP intakes during Supervised Multiple Drug Therapy (SMDT).</td>
<td>SMDT was shown to be less effective than AMDT.</td>
<td>75</td>
</tr>
<tr>
<td>2</td>
<td>2016</td>
<td>Uniform multi-drug therapy (U-MDT)</td>
<td>For Adults: RFP- 600 mg-4 weeks CLF- 300 mg-4 weeks DDS-100 mg -4 weeks CLF- 50 mg- daily For Children (10-14 yrs.) RFP- 450 mg - 4 weeks CLF-150 mg -4 weeks DDS- 50 mg - daily CLF- 50 mg– alternate days For children &lt;10 yrs: RFP:10-20 mg/kg, CLF: 1-2 mg/kg</td>
<td>3169/3437</td>
<td>(a) SPSS18.0 (b) OpenEpi</td>
<td>India (Pune, Kanpur, Tiruvannamalai and Villupuram) &amp; P. R. China (Guizhou and Yunnan)</td>
<td>For all forms of leprosy, a six-month MB-MDT regimen was recommended as U-MDT regimen.</td>
<td>(a) For both groups of patients, the regimen was determined to be acceptable and safe. (b) A shorter regimen was effective in MB patients</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DDS: 1-2 mg/kg</td>
<td>RFP- 600 mg -Once monthly CLF- 300 mg -Once monthly DDS-100 mg -Once monthly CLF- 50 mg -12 months</td>
<td>100 treated MB patients</td>
<td>Wilcoxon signed test</td>
<td>India</td>
<td>A 12-month FD-MDT for MB patients is successful and safe, having a significant operational value. A few cases of relapses may arise in the post-elimination phase.</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2018</td>
<td>Fixed duration multi-drug therapy (FD-MDT)</td>
<td>RFP- 600 mg -Once monthly CLF- 300 mg -Once monthly DDS-100 mg -Once monthly CLF- 50 mg -12 months</td>
<td>100 treated MB patients</td>
<td>Wilcoxon signed test</td>
<td>India</td>
<td>A 12-month FD-MDT for MB patients is successful and safe, having a significant operational value. A few cases of relapses may arise in the post-elimination phase.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Moxifloxacin-based regimens

|  |  | Moxifloxacin- based regimens | For mice: RPT- 10 mg MXFX- 150 mg MINO- 25 mg | 450 female Swiss mice | (a) Spearman and Karber's technique for bactericidal activity (b) Bonferroni correction for comparing dosage groups. | France | (a) Bactericidal activity of PMM> RPT. (b) The PMM combination killed 99.9% of live *M. leprae*. |
| 1 | 2000 | Rifapentine-Moxifloxacin-Minocycline (PMM) | For Patients: RPT- 600 mg MXFX- 400 mg MINO- 100 mg | 450 female Swiss mice | (a) Spearman and Karber's technique for bactericidal activity (b) Bonferroni correction for comparing dosage groups. | France | (a) Bactericidal activity of PMM> RPT. (b) The PMM combination killed 99.9% of live *M. leprae*. |

### Combination of newer anti-leprosy drugs

|  |  | Combination of newer anti-leprosy drugs | Single-dose of ROM therapy: RFP- 600 mg, OFLO- 400 mg MINO- 100 mg | 13 PB cases (BT Case) | NA | India | (a) After receiving a single dosage of ROM for 12 months, 85% of patients had no granuloma and all of them had no AFB. (b) A single dosage of ROM treatment resulted in negative histopathological activity. (ROM) is just as efficient for treating single-lesion PB leprosy patients as the conventional 6-month WHO-recommended PB-MDT regimen. |
| 1 | 1999 | Single-dose (Rifampicin, ofloxacin, and minocycline trial) (ROM-1) | Single-dose of ROM therapy: RFP- 600 mg, OFLO- 400 mg MINO- 100 mg | 13 PB cases (BT Case) | NA | India | (a) After receiving a single dosage of ROM for 12 months, 85% of patients had no granuloma and all of them had no AFB. (b) A single dosage of ROM treatment resulted in negative histopathological activity. (ROM) is just as efficient for treating single-lesion PB leprosy patients as the conventional 6-month WHO-recommended PB-MDT regimen. |
Intermittent therapy (Rifampicin, ofloxacin, and minocycline trial) (ROM)  

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Year</th>
<th>Drugs</th>
<th>Dosage given</th>
<th>Subjects recruited</th>
<th>Specimen Investigated</th>
<th>Analysis</th>
<th>Outcome</th>
<th>Remarks</th>
<th>Region of study</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2014</td>
<td>NA</td>
<td>16/21</td>
<td>NA</td>
<td>India</td>
<td>The progression and follow-up data revealed that monthly monitored</td>
<td>The regimen does not appear to raise the chance of reaction during or</td>
<td>NA</td>
<td>81</td>
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<td></td>
<td></td>
<td>prescription of ROM is effective.</td>
<td>after therapy is finished.</td>
<td></td>
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<tr>
<td>3</td>
<td>2019</td>
<td>Rifampicin and Ofloxacin (RO)</td>
<td>For MB and PB: RFP- 600 mg OFLO- 400 mg daily for only one month.</td>
<td>322/349</td>
<td>Vietnam</td>
<td>100% of patients treated with OFLO-containing regimens had shown</td>
<td>The relapse rate was quite high in patients treated with RFP and</td>
<td>Vietnam</td>
<td>82</td>
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<td></td>
<td></td>
<td>significant improvement in clinical and bacterial outcomes</td>
<td>OFLO for just one month.</td>
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</table>

Other leprosy drugs: Several contemporary drugs have been discovered, and some new combinations of drugs are also under study for treating leprosy. Table 3 represents a detailed account of such drugs and their recommended doses, along with the details of the research undertaken so far.

Table 3. Detailed profile of the other emerging leprosy drugs

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Drugs</th>
<th>Year</th>
<th>Dosage given</th>
<th>Subjects recruited</th>
<th>Specimen Investigated</th>
<th>Analysis</th>
<th>Outcome</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NTZ</td>
<td>2017</td>
<td>25 mg/ kg in infected mice- 5 days a week for four weeks in a row.</td>
<td>Female C57BL/6 mice</td>
<td>Skin and footpad tissue</td>
<td>Mann-Whitney rank sum test</td>
<td>NTZ has an inhibitory impact on <em>M. leprae</em> since it can, in a dose-</td>
<td>NTZ at 25mg/kg into <em>M. leprae</em> - infected mice exhibited anti-</td>
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<td></td>
<td>dependent manner, decrease <em>M. leprae</em> respiration.</td>
<td>mycobacterial action similar to RFP at 10 mg/kg.</td>
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</tbody>
</table>


|   | **LVFX** | **2010** | Control group: ROM regimen (RFP 600mg + OFLO 400mg + MINO 100mg)  
Study group: RLM regimen (RFP 600 mg + LVFX 500 mg + MINO 100 mg) | 72 PB patients: 36 control group, 36 study group | Blood & Slit Skin Smear | LVFX in RLM regimen resulted 75% improvement in patients, but OFLO in ROM regimen treated just 36.1% patients with P value 0.0018 | Chennai (India) |
|---|---|---|---|---|---|---|---|
|   | **MXFX** | **2008** | For patients: 400 mg of moxifloxacin as the single initial dose, followed by 7 days without treatment and then 400 mg per day from day 8 to day 56.  
For mice: Moxifloxacin 50 mg/kg five times per week | 8 untreated multibacillary leprosy patients and mice | Skin biopsies of patients and mice footpad | Spearman-Karber method to analyze results of Mouse footpad viability assays | Similar to the rate previously exclusively shown by rifampin, moxifloxacin was shown to consistently kill M. leprae in a single dose and to eliminate live bacteria within days or weeks. | Cebu, Philippines |
<p>| 4 | Bedaquiline also known as Diarylquinoline (TMC207 or R207910) | 2006 | R207910 was given as a single dose of 25 mg/kg and 100 mg/kg of body weight | Mice | Mice footpad | Method of Shepard | A single 25-mg/kg dose of R207910 killed more than 95% of the <em>M. leprae</em> bacilli that were initially implanted into the mice footpads, demonstrating the drug's potent bactericidal action against two separate isolates of the <em>M. leprae</em> bacterium. | If R207910 is used in the PMM instead of minocycline, leprosy may be treated more successfully. | Belgium, France | 85 |</p>
<table>
<thead>
<tr>
<th></th>
<th>Drug</th>
<th>Year</th>
<th>Dose/Composition</th>
<th>Species</th>
<th>Site</th>
<th>Method of Study</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>PA-82 and linezolid</td>
<td>2006</td>
<td>PA-824 and linezolid-100 mg/kg</td>
<td>Mice</td>
<td>Mice footpad</td>
<td>Method of Shepard</td>
<td>The effectiveness of PA-824 or linezolid against <em>M. leprae</em> was relatively low: a single 100-mg/kg dose did not exhibit significant bactericidal activity, and the bactericidal effect after five days of treatment was noticeably weaker, supporting the observation that PA-824 is a narrow spectrum antibiotic. For the treatment of leprosy, neither PA-824 nor linezolid make up an acceptable part of a once-monthly administered combination regimen.</td>
</tr>
<tr>
<td>6</td>
<td>Epiroprim</td>
<td>1999</td>
<td>A minimum inhibitory activity of 10 mg/l against <em>M. leprae</em></td>
<td>BALB/c mice, 6-week-old female)</td>
<td>Mice footpad</td>
<td>Student’s t-test and Fisher’s exact probability calculation</td>
<td>(a) Epiroprim completely inhibited the growth of dapsone-resistant <em>M. leprae</em> in mice footpads when added to powdered To stop the emergence of dapsone-resistant <em>M. leprae</em>, epiroprim can also be used against <em>M. leprae</em> and in conjunction</td>
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</tbody>
</table>
(a) 9 untreated MB patients: 8 males & 1 female having one lesion with 
BI greater than 4+ and MI ≥1%.  
(b) 10 female BALB/c mice 
inoculated

| 7 | SPFX | 1994 | 200 mg sparfloxacin daily for 12 weeks | Biopsy and Serum | SPFX significantly reduced MI and PGL-I titers in serum.  
(a) BI (Bacillary Index): A pre-treatment median BI of 4.25 reduced 
Bactericidal effect of SPFX 200mg (once a day) > OFLO 400mg | Philippines 87 |
with Acid Fast Bacilli in footpad viability assays to an 8-week post-treatment median BI of 3.9 with P value < 0.01
(b) MI (Morphologica l Index): After 4 weeks of therapy, no solid-staining bacilli were found in any of the patients' tests.
(c) Radiorespirometric assay: a median reduction of >99% in comparison to pre treatment values.
(d) After 12 weeks of therapy, no patient had a PGL-1 titer greater than 1 +.
<table>
<thead>
<tr>
<th>No.</th>
<th>Compound</th>
<th>Year</th>
<th>Details</th>
<th>Analysis Results</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>Fusidic acid</td>
<td>1994</td>
<td>Fusidic acid was given to patients either 500 mg/day for 12 weeks or 750 mg/day for 4 weeks continued by 500 mg/day for 8 weeks.</td>
<td>Serum and Biopsy (size that allowed five 6-mm skin punch biopsy) (a) Wilcoxon signed rank test to analyze BI, MI &amp; radiorespirometry data (b) Spearman-Karber method to analyze results of Mouse footpad viability assays (a) Median BI remained unaltered (4.7) before and after 8 weeks of treatment. (b) Median MI decreased progressively from 2 weeks to 8 weeks. Fusidic acid is effective in cases of human leprosy at the dosages used, albeit it is not promptly bactericidal.</td>
<td>Philippines</td>
</tr>
<tr>
<td>9</td>
<td>Amoxicillin with potassium clavulanate (or Beta-lactam antibiotics)</td>
<td>1991</td>
<td>200-600mg/kg of dose of amoxicillin with potassium clavulanate</td>
<td>(a) Method of Shepard (b) Spearman-Karber method (a) For <em>M. leprae</em>, the mixture of amoxicillin and clavulanic acid is bactericidal. (b) The combination of amoxicillin and clavulanic acid produced bactericidal activity for <em>M. leprae</em> comparable to that previously observed for dapsone, the cornerstone of contemporary leprosy treatment. Clinical investigations suggest that amoxicillin plus clavulanic acid is unlikely to be sufficiently bactericidal in humans to serve as the second bactericidal agent required to cure lepromatous leprosy.</td>
<td>Louisiana</td>
</tr>
<tr>
<td>Ansamycins (R-76-1 and DL 473 are two newer drugs)</td>
<td>1986</td>
<td>(a) Female Swiss albino mice (b) 20 LL patients</td>
<td>Spleen &amp; footpad of mice and biopsies of patients</td>
<td>(a) Two-tailed Student t-test/therapeutic index: for comparison of the quantity of <em>M. leprae</em> murium extracted from each group's spleen (b) Spearman-Karber method: to analyze significance of the variations in median infectious dose values. (c) In comparison to the other ansamycins, R-76-1 was more effective against the majority of cultivable mycobacteria, including <em>M. leprae</em> murium. (b) R-76-1 was around three times more efficient than RMP. (c) The anti-<em>M. leprae</em> murium activity of DL 473 was longer-lasting than that of RMP.</td>
<td>China</td>
</tr>
</tbody>
</table>

*Abbreviations: Levofloxacin (LVFX), Sparfloxacin (SPFX), Nitazoxanide (NTZ), and Moxifloxacin (MXFX).*
Availability of new tools and their scope in elimination

The administration of MDT to newly diagnosed leprosy cases continues to be the cornerstone of leprosy treatment. The ineffective MDT approach requires a novel method suited to the current epidemiological scenario. Failure of MDT to eliminate leprosy is not due to the ineffectiveness, but is due to long incubation period and skin signs that are often difficult for an inexperienced diagnostician. This results in a persistent infectious population giving rise to cross infection before MDT treatment is administered. This dependence on skilled diagnosis could receive greater emphasis as could social factors making cross infection more likely such as overcrowding and poor nutrition particularly to explain why so many children present with advanced disease. One skill that ensures diagnosis is skin scraping. With HIV it became unpopular, but the Bombay Leprosy Centre finds it still very valuable.

In general population, the possibility of transmitting leprosy is quite low. Nonetheless, direct contact with newly diagnosed, untreated individuals provides highest risk. Interactions within home will increase new cases. When implementing contact tracing into practice, practical and ethical factors must be taken into account. In recent years, advancements in chemotherapy and immunoprophylaxis for leprosy prevention have been made, with the main beneficiaries of these therapies being the close relatives \textsuperscript{91}. Rifampicin chemoprophylaxis with a single dose is cost-effective, but additional research is required to evaluate its applicability. Control efforts would greatly benefit from knowing if leprosy contacts have \textit{M.leprae} infection and more importantly, whether they are prone to getting the disease. In this situation, preventive treatment could be offered. It is challenging to develop tests based on immunological biomarkers that can distinguish between healthy individuals and those who are unwell. It is challenging to develop immunological biomarker-based assays that can distinguish between healthy individuals and cases \textsuperscript{92}.

Currently, a significant amount of effort is being devoted to the development of specific T-cell diagnostic assays and the evaluation of their accuracy and utility. Depending on the results of one or more of these tests, the selected intervention for the contact may be MDT, chemoprophylaxis, or immunoprophylaxis. Modeling studies indicate that all three interventions—chemoprophylaxis, BCG
vaccination, and diagnosis of sub-clinical infection and treatment—will reduce the prevalence of leprosy in the general population if implemented routinely in household contacts of leprosy cases.\(^{93}\)

**Vaccines**

Vaccines for leprosy should generate a robust, long-lasting T cell response against *M.leprae*, consequently protecting against the disease and reducing its transmission rate. To combat leprosy, sub-unit vaccinations would be more focused and targeted and have long-lasting effects. Since the *M. leprae* genome sequencing was completed in 2001, the production of recombinant antigens has become easier. It is believed that the cellularity of a draining sub-unit lymph node (DLN) may be utilized to assess the level of infection.\(^{91}\) Antigen identification is critical for effective vaccination. With the support of the American Leprosy Missions, the Infectious Disease Research Institute (IDRI), Seattle, U.S., has identified many antigens recognized by PB cases that in turn trigger IFN-\(\alpha\) production. Increased T cell concentration indicated that the DLN cellularity at the infection site had increased.\(^{94}\) However, these alterations were not seen when dead *M.leprae* was injected and infection was treated with rifampicin. A recent study demonstrated potent antigen-specific Th1 responses which lower disease-related inflammation, but did not reduce bacterial burden.

Leprosy is also associated with defective cell-mediated immunity (CMI) which decreases from PB to MB. Although MDT kills bacilli, it has no role in enhancing CMI. It cannot prevent the susceptibility to acquired infection nor effectively remove dead bacilli from the body, rendering the individual to dead bacilli–related complications like hypersensitivity reaction. To enhance the CMI of host, various vaccines have been explored. Vaccine trials have utilized live or killed whole mycobacterium, including bacille Calmette-Guérin (BCG), ICRC (Indian Cancer Research Centre) bacilli, and MIP (*Mycobacterium indicus pranii*) formerly known as *Mycobacterium w* (*M.w*) developed from either heat-killed whole *M.leprae* alone or in combination with live BCG have been considered safe.\(^{95}\) Gupte and colleagues revealed that BCG/*M.leprae* offered 64%, ICRC bacilli 65.5%, *M.w* 25.7%, and BCG alone 34.1% protection. In contrast to previous studies of Venezuela and Malawi, the south India experiment showed that both ICRC and BCG/*M. leprae* vaccines met the criteria for public health.\(^{91}\)

Sharma and colleagues\(^{96}\) published the outcome of double-blind immunoprophylactic study of *M.w* vaccine conducted in Kanpur Dehat. At the culmination of the first, second, and third follow-up periods, protective efficacies of 43%, 31%, and 3% were detected. The use of *M.habana* as a vaccine
has also been suggested due to its protective effects in mice and its ability to stimulate lepromin reactions in monkeys.\textsuperscript{97,98} After receiving the \textit{M. habana} vaccine, 100\% of LL cases and their household contacts who tested negative for lepromin had a consistent lepromin conversion, while 100\% of those who tested positive for lepromin experienced an increase in lepromin reactivity.\textsuperscript{99} Enhanced lepromin reactivity indicated that \textit{M. habana} vaccination promoted specific CMI against \textit{M. leprae}.

\textbf{Future perspectives in leprosy treatment}

Leprosy has a significant worldwide frequency, and patients often suffer long-term repercussions. Microbiologically, MDT may cure leprosy; nevertheless, the treatment is insufficient to prevent nerve damage and other complications associated with leprosy reactions. Despite the efforts of statisticians, it is important to remember that disabilities and dysfunction of many patients persist after therapy. In the past, cases of leprosy recurrence have also been reported and reactional episodes raise additional treatment-related concerns. Antibiotic-resistant microorganisms are also a serious threat to the present treatment methods. Next-generation research is needed to define and improve the criteria for treatment failure after WHO’s MDT and predict the elements that lead to treatment non-response. Extending anti-leprosy therapy in nonresponsive patients compared to the standard multidrug multibacillary regimen should be intriguing (MDT-MBR25).

Vaccines such as BCG, LepVax, and MIP have been utilized to reduce the challenges of leprosy therapy. The inclusion of vaccination in MDT treatment is recommended for future clinical assessment.\textsuperscript{100} A more practical method for monitoring the disease progression in a shorter time is to focus on early diagnosis of leprosy by employing leprosy biomarkers and therapies on the most susceptible people (contacts of highly infected cases), many of whom may already be infected with \textit{M. leprae}. However, success as a chemo- and immuno-therapeutic intervention after exposure bodes well for the transition from therapeutic to preventative administration in a larger population. Leprosy treatment efficacy may be improved by using nano-emulsions (less water-soluble medicines) for effective medication absorption.

\textbf{Conclusion}

\textit{M. leprae} infection is curable using anti-leprosy therapies. It undergoes genome reduction, drug resistance, and environmental adaptation process, which has made it necessary for continuous hunt
of new drugs over time. Since the diagnosis of leprosy has always posed a challenge in the people of non-endemic regions, its transmission to these regions is highly suspected through travelers from endemic regions. On a global scale, a combination of MDT and appropriate vaccinations can be utilized to reduce disease transmission among travelers returning from endemic regions. As a result of this data, several other assays for identification of drug resistance in \textit{M.leprae} have been developed. Currently, laboratories all around the world utilize PCR/direct DNA sequencing to identify \textit{M.leprae} drug resistance strains. These innovative assays are anticipated to develop into low-cost, point-of-care diagnostic tools for tracking drug resistance in leprosy, which is urgently required.

**Conflict of Interest**

No conflict of interest.

**Author Contributions**

All authors have contributed equally to the conceptualization and development of the content.

SG & SS: paper extraction & writing

DS: editing

DB, ND, AG & VKG: revised and finalized the manuscript

The final version of the manuscript has been read and approved by all authors.

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