Increscent journey of anti-leprosy drug development

Sakshi Gautam[1,2], Devesh Sharma[1], Sakshi Singh[1], Nirmala Deo[1], Anjana Goel[2], Vivek Kumar Gupta[1] and Deepa Bisht[1,*]

[1]Department of Biochemistry, ICMR-National JALMA Institute for Leprosy and Other Mycobacterial Diseases, Tajganj 282 001, India
[2]Department of Biotechnology, GLA University, NH-2, Mathura-Delhi Road, Mathura 281 406, India

Leprosy, a chronic granulomatous disease generally caused by *Mycobacterium leprae* and *Mycobacterium lepromatosis*, remains a serious public health concern, particularly in developing countries. With the introduction of multi-drug therapy (MDT) by the World Health Organization in 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 challenging to cultivate *M. leprae* in a laboratory environment on a synthetic medium. Thus, studies on animal models have assisted in evaluating 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 the research and development of anti-leprosy drugs.

Keywords: Animal models, antibiotic, drugs-mode of action, *Mycobacterium leprae*, pharmacokinetics, vaccine.

THE etiological agents of leprosy, *Mycobacterium leprae* and *Mycobacterium 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. The inability to cultivate *M. leprae in vitro* has led to the use of animal models to test novel medications, vaccines and fundamental pathogenesis mechanisms[2].

*For correspondence. (e-mail: abd1109@rediffmail.com)

The incidence of leprosy has decreased worldwide since the 1980s, with the introduction of multi-drug therapy (MDT) by the World Health Organization (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 spread by untreated patients[3]. Previous studies have 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. Developing more pharmacological and immunotherapeutic strategies to protect neurologic function is necessary as neuropathy still poses a challenge, particularly if diagnosis and treatment are deferred[9].

Current findings by Yamaguchi *et al.*[10] revealed that fluoroquinolones DC-159a and sitafloxacin are more effective than moxifloxacin against wild-type and mutant *M. leprae* DNA gyrases. 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 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.*[14] 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 programme[14].

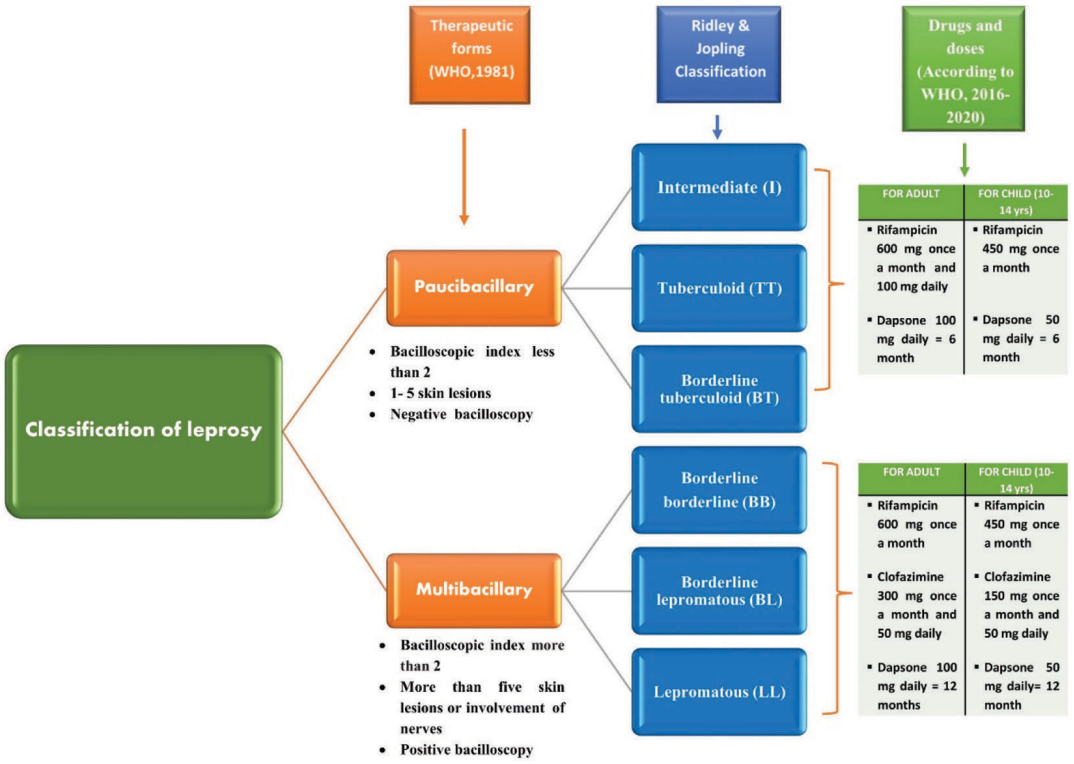

Figure 1. A schematic representation of classification of leprosy given by WHO, and Ridley and Jopling[12].

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, cataloguing drug-resistant strains, and conducting basic immunological studies, including vaccine efficacy testing. Johnstone[15] 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 for dozens or hundreds of mice. The usual time for MFP studies to provide data on 'culture and sensitivity' for *M. leprae* is 12 months or more. It enabled researchers to assess the effectiveness of anti-*M. leprae* medications before starting a clinical investigation and was still the best approach available 40–50 years ago[16].

Apart from humans, armadillos served as a model for *M. leprae* infection in 1971 (ref. 17). Many functional, physiological and anatomical features of armadillo's leprosy were comparable to human leprosy. Armadillos also exhibited the whole clinical spectrum of leprosy and severe peripheral nerve damage. This knowledge improvement has permitted the testing of novel therapeutic and diagnostic regimens in armadillos that have provided new insights into the oldest known neurodegenerative disease[18]. The liver, spleen, lymph nodes, lips, tongue, nose, nasal mucosa, skin, bone marrow, eyes, lungs and nerves are among the organs where *M. leprae*-infected macrophages have been shown to infiltrate the armadillo[19]. Using the leprae-specific repetitive element (RLEP) of DNA extracted from an armadillo's ear, liver and lungs, Vera-Cabrera *et al.*[20] established the presence of *M. leprae* in tissues by polymerase chain reaction (PCR) testing[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 *M. leprae* infection. Mice vaccinated with the LepVax vaccine had about 85% lower bacterial loads than those seen in animals 12 months later. A study found that when LepVax was given to armadillos exposed to *M. leprae*, it prevented and slowed down the damage to the motor and sensory nerves[21]. Adams *et al.*[22] recently discussed *M. leprae* susceptibility and drug resistance, focusing on *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 *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, the 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, strychnine, sodium salicylate, carbolic acid, various kinds of baths, radium, electric current, X-ray, and surgical procedures such as nerve stretching, bleeding and ulcer removal[23] and so on.

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 internally[24]. Cyclopentenyl fatty acids in seed oil were linked to its antileprotic properties[25]. 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 medication[24]. In 1941, Promin was the first sulfone medication used to treat leprosy. Dr Guy Faget of Carville, Louisiana, was the first to test it[26].

First-line drugs

Since 1982, WHO has recommended Clofazimine, Rifampicin and Dapsone as the first-line medications for leprosy[13]. They are the cornerstone antibiotics of MDT.

Dapsone

Compound name: 4,4′-diaminodiphenylsulfone. The usage of dapsone (DDS) was spurred by the side effect of promin. It possesses antimicrobial/antiprotozoal and anti-inflammatory properties[27].

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 biosynthesis[28].

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 impaired[29]. 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 h (refs 30, 31). Hemolytic anaemia and dapsone hypersensitivity syndrome (DHS) are the consequences that emerge from hydroxylamine (a toxic metabolite of dapsone)[32]. Peak serum concentrations are attained in 2–8 h (ref. 33), and dosage recommendations are 1–2 mg/kg (ref. 34). 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].

Resistance to dapsone: Resistance to dapsone is caused due to mutation in codon 55 of the *folP* gene prompted by substituting leucine with proline[35]. According to Nisha *et al.*[36], CID21480113 (4-(2-fluorophenylsulfonyl) benzenamine) can be developed as a medication for dapsone-resistant leprosy patients[36].

Rifampicin (RFP) or rifampin

Compound name: 3-(4-methyl-1-piperazinyl)-imino-methyl-rifamycin.

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 *M. leprae* does not replicate[37].

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 h. Serum peak concentrations of 10 g/ml are observed between 1 and 2 h. A single dosage of 600 mg of rifampin kills 92.1% of the total bacilli[38].

Resistance to rifampicin: Rifampin resistance in *M. leprae* is caused by a missense mutation in the *rpoB* gene, which codes for the β-sub-unit 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)[39]. Richardus *et al.*[40] reported that single-dose rifampicin (SDR) for post-exposure prophylaxis was safe and interpreted that it could be implemented into various leprosy control programmes.

Clofazimine

Compound name: 3-(*p*-chloroanilino)-10-(*p*-chlorophenyl)-2,10]-dihydro (isopropylimino)-phenazine). Clofazimine (CLF), initially known as B663, is a lipophilic riminophenazine antibiotic with anti-mycobacterial action and anti-inflammatory properties[41]. An important feature of riminophenazine is the phenazine nucleus with an alkylimino and phenyl substituent necessary for antibacterial activity[42]. Accumulation of CLF crystals in the colon can lead to fatal and severe CLF-induced enteropathy and skin pigmentation[43].

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_2O_2 and super oxide, which have antibacterial characteristics, are generated by CLF via redox cycling. It was later discovered that the bactericidal efficacy of CLF was due to its interaction with the bacterial membrane phospholipids to generate antimicrobial lysophospholipids, which might result from the combined membrane destabilizing effects of both CLF and lysophospholipids, interfering with K^+ uptake and eventually adenosine triphosphate (ATP) production[44]. Although CLF's anti-inflammatory effects are probably due to suppressing T lymphocyte activation and proliferation, it might also block the function of the Kv1.3 potassium channel[45].

Clinical pharmacokinetics profile: Oral absorption of CLF is gradual and dose-dependent[33]. According to Feng *et al.*[46], metabolite I was the result of a hydrolytic dehalogenation process, whereas metabolite II was the result of hydrolytic deamination followed by glucuronidation, and its half-life varied, ranging from 10 to 70 days in single and multiple-dose studies. Its peak plasma concentration was 407.6 ng/g between 4 and 8 h, after a 200 mg oral dosage was administered 10 min after breakfast. When the dosage is raised, the drug's faecal excretion rises, and approximately 1% of the dosage's metabolites are excreted in urine[33]. Recently, Yuan *et al.*[47] suggested that CLF might be important in controlling future coronavirus outbreaks.

Second-line drugs

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

Fluoroquinolones

Fluoroquinolones (FQs), viz. pefloxacin, ofloxacin, norfloxacin, ciprofloxacin and enoxacin, are most well-investigated for their antibacterial activity against gram-negative and gram-positive microorganisms[33]. For PB individuals with a single lesion, ofloxacin is recommended in current MDT regimens[48].

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, killing certain bacteria within hours[49].

Clinical pharmacokinetic profile: The absorption rate of ofloxacin (OFLO) is around 98%. It is mostly eliminated unaltered by the kidneys, and its half-life is approximately 5–8 h, while pefloxacin is 10–12 h. After 2 h, serum concentrations peak at 2.9 g/ml (refs 50, 51). Except for ofloxacin, all fluoroquinolones are metabolized by the liver[51]. Moxifloxacin has strong immunomodulatory characteristics, like suppression of tumour 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].

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.

Minocycline

Compound name: 7-dimethylamino-6-dimethyl-6-deoxytetracycline. Minocycline (MINO), 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 with DDS, RFP and clarithromycin[56].

Mode of action: Minocycline's mechanism of action against *M. leprae* is unknown, although it is presumed to be identical to 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 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].

Clinical pharmacokinetics profile: Absorption of minocycline in the jejunum ranges from 95% to 100% (ref. 59), and it metabolizes in the liver. Mass spectral studies show it is metabolized into 9-hydroxyminocycline and two other mono-N-demethylated derivatives[60]. Within 2 h 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 was estimated to be between 6 and 11 h. The recommended daily dose is 100 mg. It is mostly excreted in the faeces and at a modest rate (5–12%) in urine[59].

Clarithromycin

Compound name: 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 the 6th position of macrolide ring[61]. It has anti-inflammatory actions and

Table 1. Anti-leprosy medicines' side effects

Drugs	Severity	Incidence	Reference
Dapsone	Fever, hepatitis, skin reactions, headache	Very common (>80%)	83
	Lymphadenopathy, pruritus, leukocytosis, anemia, eosinophilia	Common (50–80%)	
	Mucosal involvement, exfoliative dermatitis splenomegaly, nausea and vomiting, atypical lymphocytosis, hemolytic anemia	Less common (10–50%)	
Rifampicin	Cutaneous problem	Uncommon (≤5%)	84
	Gastrointestinal manifestations	Variable	
	Hepatitis	Common (≤1%)	
	Thrombocytopenic purpura	Very uncommon	
	Hemolytic anemia, shortness of breath, renal failure	Rare	
	Flu Syndrome: fever, chills, and sometimes headache, dizziness, and bone pain	Uncommon during the initial weeks	
Clofazimine	Skin reactions	NA	85
	Gastrointestinal manifestation		
	Eye toxicity		
Fluoroquinolones	Gastrointestinal symptoms	15.3%	86
	Cutaneous symptoms	20.3%	
	Musculoskeletal problems	6.8%	
	Central nervous system problems	11.9%	
	Peripheral nervous system problems	6.8%	
	Cardiovascular problem	18.6%	
	Other	20.3%	
Minocycline	Headache	(up to 23%) very common	87
	Gastrointestinal symptoms	Common (1–10%)	
Clarithromycin	Gastrointestinal manifestations, Cutaneous problem	NA	88

*NA, Not available.

modulatory effects on cytokines and chemokine production, while it has immuno-modulatory effects on inflammatory cells, fibroblasts and epithelial cells[62].

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 (ref. 54).

Clinical pharmacokinetics profile: Clarithromycin is readily absorbed from the gastrointestinal tract, but its systemic availability is decreased due to first-pass metabolism (roughly 55%). It degrades quickly and is transformed into an active 14-hydroxy (R) metabolite with a half-life of 6–7 h. The drug concentration peaks at 1 g/ml after 1–4 h and is mostly eliminated in urine with the parent component[51].

Resistance to clarithromycin: Resistance to macrolides appears to be related to a 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[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.

Multi-drug therapy

Initially, MDT was prescribed for two years or until the smear of an MB case tested negative[13,64]. 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[65]. WHO also recommended a single-dose regimen for individuals with just one PB lesion[66]. Despite this development, new-case detection rates are still steady in Brazil and India, with the 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[67], the novel multi-targeted therapy for leprosy aims to reduce drug resistance and increase therapeutic efficacy. Multi-targeted therapy aims 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. An overview of the drug discovery is represented in Figure 2. There are three main stages here: The infection stage, when the disease spreads to a healthy person; the observation stage, when main symptoms develop and are observed; and the experimental stage, when animals and the person affected with

Table 2. Detailed profile of drug therapies including (i) Multi-drug therapy, (ii) Combination of newer anti-leprosy drugs, (iii) Moxifloxacin-based regimens

	Year	Therapy	Drug recommendation	No. of subjects that completed treatment/ total no. of subjects	Statistical analysis	Region of study	Outcomes	Remarks	Reference
Multi-drug treatment	2004	Accompanied multi-drug therapy (A-MDT)	NA	962/1000	NA	Madagascar	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 (S-MDT)	SMDT was shown to be less effective than A-MDT	69
	2016	Uniform multi-drug therapy (U-MDT)	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 years) RFP: 450 mg – 4 weeks CLF: 150 mg – 4 weeks DDS: 50 mg – daily CLF: 50 mg – alternate days For children <10 years RFP: 10–20 mg/kg CLF: 1–2 mg/kg DDS: 1–2 mg/kg	3169/3437	(a) SPSS18.0 (b) OpenEpi	India (Pune, Kanpur, Tiruvannamalai and Villupuram) and P.R. China (Guizhou and Yunnan)	For all forms of leprosy, a six-month MB-MDT regimen was recommended as U-MDT regimen	(a) For both groups of patients, the regimen was determined to be acceptable and safe. (b) A shorter regimen was effective in MB patients	68
	2018	Fixed duration multi-drug therapy (FD-MDT)	RFP: 600 mg – once monthly CLF: 300 mg – once monthly DDS: 100 mg – once monthly CLF: 50 mg – 12 months	100 treated MB patients	Wilcoxon signed test	India	A 12-month FD-MDT for MB patients was successful and safe, having a significant operational value	A few cases of relapses may arise in the post-elimination phase	89
Moxifloxacin-based regimens	2000	Rifapentine-moxifloxacin-minocycline (PMM)	For mice RPT: 10 mg MXFX: 150 mg MINO: 25 mg 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.*	One dose of PMM is sufficient for the treatment of PB multiple-lesion patients and is more effective than ROM for the treatment of PB single-lesion leprosy	90

(*Contd*)

Table 2. (*Contd*)

	Year	Therapy	Drug recommendation	No. of subjects that completed treatment/ total no. of subjects	Statistical analysis	Region of study	Outcomes	Remarks	Reference
Combination of newer anti-leprosy drugs	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	91
	2014	Intermittent therapy (Rifampicin, ofloxacin and minocycline trial) (ROM)	NA	16/21	NA	India	The progression and follow-up data revealed that monthly monitored prescription of ROM was effective	The regimen does not appear to raise the chance of reaction during or after therapy is finished	92
	2019	Rifampicin and Ofloxacin (RO)	For MB and PB RFP: 600 mg OFLO: 400 mg daily for only one month	322/349	SPSS version 13, SPSS Inc. *P*-value <0.05	Vietnam	100% of patients treated with OFLO-containing regimens, had shown significant improvement in clinical and bacterial outcomes	The relapse rate was quite high in patients treated with RFP and OFLO for just one month.	93

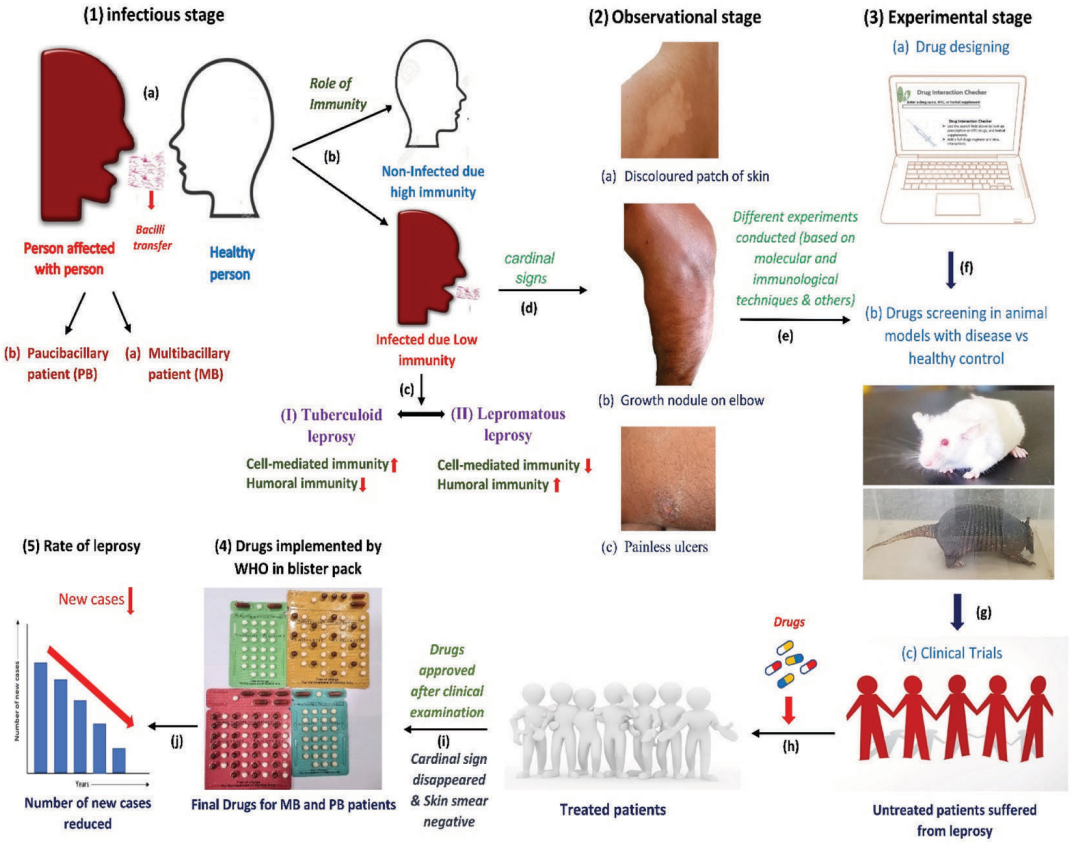

Figure 2. Schematic diagram of drug design for leprosy treatment.

leprosy are subjected to treatment. After the investigations and validation of a particular drug, the WHO approves it.

MDT regimens for treating 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 misclassification of leprosy cases. According to Manickam *et al.*[68], 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 recommended accompanied-MDT (A-MDT) to aid populations who live in remote border regions, urban slums, and areas of civil unrest, as well as migrant workers[69]. 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[70].

In treating leprosy, other drugs with distinct modes of action have been introduced. These drugs inhibit various molecular processes like replication, transcription and translation. In the 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 (isobutyl piperazinyl rifampicin SV) inhibit the DNA-dependent RNA polymerase of bacteria. Fusidic acid inhibits the translocation factor G during protein synthesis[51]. Beta-lactam antibiotics cephaloridine, cefuroxime and amoxicillin plus clavulanic acid inhibit the formation of the 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*[71].

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.

New tools and their scope in elimination of leprosy

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

Table 3. Detailed profile of the other emerging leprosy drugs

Drugs	Year	Dosage given	Subjects recruited	Specimen investigated	Analysis	Outcome	Remarks	Region of study	Reference
NTZ	2017	25 mg/kg in infected mice – 5 days a week for four weeks in a row	Female C57BL/6 mice	Skin and footpad tissue	Mann–Whitney rank sum test	NTZ has an inhibitory impact on *M. leprae* since it can, in a dose-dependent manner, decrease *M. leprae* respiration	NTZ at 25 mg/kg into *M. leprae*-infected mice exhibited anti-mycobacterial action similar to RFP at 10 mg/kg	America	71
LVFX	2010	Control group: ROM regimen (RFP 600 mg + OFLO 400 mg + MINO 100 mg) Study group: RLM regimen (RFP 600 mg + LVFX 500 mg + MINO 100 mg)	72 PB patients: 36 control group, 36 study group	Blood and slit skin smear	Fisher's exact test	LVFX in RLM regimen resulted 75% improvement in patients, but OFLO in ROM regimen treated just 36.1% patients with *P* value 0.0018	Success rate of LVFX (500 mg) > OFLO (400 mg) in leprosy treatment	Chennai (India)	94
MXFX	2008	For patients: 400 mg of MXFX 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: MXFX 50 mg/kg five times per week	8 untreated MB leprosy patients and mice	Skin biopsies of patients and mice footpad	Spearman–Karber method to analyse results of mouse footpad viability assays	(a) At days 28 and 56, no viable *M. leprae* was found in any of the 8 patients. (b) MXFX was found to clear skin lesions regularly and uniformly quickly (possibly as a result of MXFX's anti-inflammatory and immunomodulating effects), to have no harmful side effects, and to be rapidly bactericidal for *M. leprae* (bactericidal activity displayed earlier only by RFP)	Similar to the rate previously exclusively shown by RFP, MXFX was also observed to consistently kill *M. leprae* in a single dose and to eliminate live bacteria within days or weeks	Cebu, Philippines	95
Bedaquiline also known as Diarylquinoline (TMC207or 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 *M. leprae* bacilli that were initially implanted into the mice footpads, demonstrating the drug's potent bactericidal action against two separate isolates of the *M. leprae* bacterium	If R207910 is used in the PMM instead of MINO, leprosy may be treated more successfully	Belgium, France	96

(*Contd*)

Table 3. (*Contd*)

Drugs	Year	Dosage given	Subjects recruited	Specimen investigated	Analysis	Outcome	Remarks	Region of study	Reference
PA-84 and linezolid	2006	PA-824 and linezolid 100 mg/kg	Mice	Mice footpad	Method of Shepard	The effectiveness of PA-824 or linezolid against *M. leprae* 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 monthly once administered combination regimen	Belgium, France	96
Epiroprim	1999	A minimum inhibitory activity of 10 mg/l against *M. leprae*	BALB/c mice, 6-week-old female	Mice footpad	Student's *t*-test and Fisher's exact probability calculation	(a) Epiroprim completely inhibited the growth of dapsone-resistant *M. leprae* in mice footpads when added to powdered mouse diet at a dose of 0.05%; the outcomes were bactericidal ($P < 0.01$). (b) The concentrations of epiroprim and DDS needed to achieve complete inhibition when combined, were 0.01% (reduction of 80% when administered alone) and 0.0005% (reduction of 95%, $P < 0.01$) respectively	To stop the emergence of DDS-resistant *M. leprae*, epiroprim can also be used against *M. leprae* and in conjunction with DDS	Melbourne, USA	97
SPFX	1994	200 mg SPFX daily for 12 weeks	(a) 9 untreated MB patients: 8 males and 1 female having one lesion with bacillary index (BI) greater than 4+ and morphological index (MI) ≥1%. (b) 10 female BALB/c mice inoculated with acid fast bacilli in footpad	Biopsy and Serum	(a) Wilcoxon signed rank test to analyse BI, MI and radiorespirometry data (b) Spearman–Karber method to analyse results of mouse footpad viability assays	SPFX significantly reduced MI and PGL-I titers in serum. (a) BI: A pre-treatment median BI of 4.25 reduced to an 8-week post-treatment median BI of 3.9 with P value < 0.01 (b) MI: 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 +.	Bactericidal effect of SPFX 200 mg (once a day) > OFLO 400 mg	Philippines	98

(*Contd*)

Table 3. (*Contd*)

Drugs	Year	Dosage given	Subjects recruited	Specimen investigated	Analysis	Outcome	Remarks	Region of study	Reference
Fusidic acid	1994	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	9 LL patients: 7 males and 2 females with BI 4 + and MI ≥1%.	Serum and Biopsy (size that allowed five 6 mm skin punch biopsy)	(a) Wilcoxon signed rank test to analyse BI, MI and radiore-spirometry data (b) Spearman–Karber method to analyse 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	Philippines	99
Amoxicillin with potassium clavulanate (or beta-lactam antibiotics)	1991	200–600 mg/kg of dose of amoxicillin with potassium clavulanate	BALB/c mice	Mice footpad	(a) Method of Shepard (b) Spearman–Karber method	(a) For *M. leprae*, the mixture of amoxicillin and clavulanic acid is bactericidal. (b) The combination of amoxicillin and clavulanic acid produced bactericidal activity for *M. leprae* comparable to that previously observed for DDS, 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	Louisiana	100
Ansamycins (R-76-1 and DL 473 are two newer drugs)	1986	R-76-1: a daily dosage of 150 mg for from 6 to 18 months, DL473: 5–10 mg/kg	(a) Female Swiss albino mice (b) 20 LL patients	Spleen and footpad of mice and biopsies of patients	(a) Two-tailed Student *t*-test/ therapeutic index: for comparison of the quantity of *M. leprae murium* extracted from each group's spleen (b) Spearman–Karber method: to analyse significance of the variations in median infectious dose values	(a) In comparison to the other ansamycins, R-76-1 was more effective against the majority of cultivable mycobacteria, including *M. leprae murium*. (b) R-76-1 was around three times more efficient than RMP. (c) The anti-*M. leprae murium* activity of DL 473 was longer-lasting than that of RMP.	R-76-1 was roughly 8 times more active *in vitro* than RMP in terms of minimal inhibitory concentrations against a variety of cultivable mycobacteria, whereas DL 473 was just marginally more active than RMP	China	101

LVFX, Levofloxacin; SPFX, Sparfloxacin; NTZ, Nitazoxanide and MXFX, Moxifloxacin.

MDT to eliminate leprosy is not due to the ineffectiveness but the 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 the general population, the possibility of transmitting leprosy is quite low. Nonetheless, direct contact with newly diagnosed, untreated individuals provides the highest risk. Interactions within the home will increase new cases. When implementing contact tracing in 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 close relatives[72]. Rifampicin chemoprophylaxis with a single dose is cost-effective, but additional research is required to evaluate its applicability. Control efforts will greatly benefit from knowing if leprosy contacts have *M. leprae* infection and, more importantly, whether they are prone to getting the disease. In this situation, preventive treatment could be offered. It is also challenging to develop tests based on immunological biomarkers that can distinguish between healthy and unwell individuals. It is also challenging to develop immunological biomarker-based assays that can distinguish between healthy individuals and affected cases[73].

Currently, a significant amount of effort is being devoted to developing specific T-cell diagnostic assays and evaluating their accuracy and utility. Depending on the results of one or more of these tests, the selected intervention for the contact could be MDT, chemoprophylaxis, or immunoprophylaxis. Modelling studies indicate that all three interventions – chemoprophylaxis, bacille Calmette-Guérin (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[74].

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, 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[72]. Antigen identification is critical for effective vaccination. With the support of the American Leprosy Missions, the Infectious Disease Research Institute, Seattle, US, has identified many antigens recognized by PB cases that, in turn, trigger alpha interferon (IFN-α) production. Increased T-cell concentration indicated that the DLN cellularity at the infection site had increased[75]. However, these alterations were not seen when dead *M. leprae* was injected, and the infection was treated with rifampicin. A recent study demonstrated potent antigen-specific Th1 responses, which lowered disease-related inflammation but not reducing 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 the host, various vaccines have been explored. Vaccine trials have utilized live or killed whole mycobacterium, including 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[76]. Gupte *et al.*[77] 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 both ICRC and BCG/*M. leprae* vaccines met the criteria for public health[72].

Sharma *et al*.[77] published the outcome of a double-blind immunoprophylactic study of *M.w* vaccine conducted in Kanpur Dehat, India. At the culmination of the first, second, and third follow-up periods, protective efficacies of 43%, 31% and 3% were detected. The use of *Mycobacterium habana* as a vaccine has also been suggested due to its protective effects in mice and its ability to stimulate lepromin reactions in monkeys[78,79]. After receiving the *M. habana* vaccine, 100% of LL cases and their household contacts who tested negative for lepromin had a consistent conversion, while 100% of those who tested positive for lepromin experienced an increase in lepromin reactivity[80]. Enhanced lepromin reactivity indicated that *M. habana* vaccination promoted specific CMI against *M. leprae*.

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 the disabilities and dysfunction of many patients persist even 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 non-responsive patients compared to the standard multi-drug multi-bacillary regimen (MDT-MBR25) should be intriguing.

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[81]. 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 *M. leprae.* However, success as a chemo- and immuno-therapeutic intervention after exposure bode well for transitioning from therapeutic to preventative administration in a larger population. Leprosy treatment efficacy may be improved using nano-emulsions (less water-soluble medicines) for effective medication absorption.

Conclusion

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 the continuous hunt for 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 travellers from endemic regions. On a global scale, a combination of MDT and appropriate vaccinations can be utilized to reduce disease transmission among travellers returning from endemic regions. As a result of this data, several other assays for identifying drug resistance in *M. leprae* have been developed. Currently, laboratories all around the world utilize PCR/direct DNA sequencing to identify *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: The authors declare no conflict of interest.

1. Han, X. Y., Sizer, K. C., Velarde-Félix, J. S., Frias-Castro, L. O. and Vargas-Ocampo, F., The leprosy agents *Mycobacterium lepromatosis* and *Mycobacterium leprae* in Mexico. *Int. J. Dermatol.*, 2012, **51**, 952–959.
2. Gillis, T. P., *Mycobacterium leprae.* In *Molecular Medical Microbiologyt*, Elsevier Ltd, London, 2014, 2nd edn.
3. Palit, A. and Kar, H. K., Prevention of transmission of leprosy: the current scenario. *Indian J. Dermatol. Venereol. Leprol.*, 2020, **86**, 115–123.
4. Gillis, T. P. and Williams, D. L., Dapsone resistance in *Mycobacterium leprae*. *Lepr. Rev.*, 2000, **71**, 91–95.
5. Honore, N. C. S., Molecular basis of Rifampin resistance in *Mycobacterium leprae*. *Antimicrob. Agents Chemother.*, 1993, **37**, 414–418.
6. Veziris, N. *et al.*, Resistance of *M. leprae* to Quinolones: a question of relativity? *PLoS Negl. Trop. Dis.*, 2013, **7**, 1–5.
7. Matsuoka, M., Global surveillance system to monitor the development of drug resistance in Mycobacterium leprae. *Res. Rep. Trop. Med.*, 2015, **6**, 75–83.
8. Pandhi, D. and Chhabra, N., New insights in the pathogenesis of type 1 and type 2 lepra reaction. *Indian J. Dermatol. Venereol. Leprol.*, 2013, **79**, 739–749.
9. Ebenezer, G. J. and Scollard, D. M., Treatment and evaluation advances in leprosy neuropathy. *Neurotherapeutics*, 2021, **18**, 2337–2350.
10. Yamaguchi, T., Yokoyama, K., Nakajima, C. and Suzuki, Y., DC-159a shows inhibitory activity against DNA Gyrases of *Mycobacterium leprae*. *PLoS Negl. Trop. Dis.*, 2016, **10**, 1–13.
11. Gautam, S., Sharma, D., Goel, A., Patil, S. A. and Bisht, D., Insights into *Mycobacterium leprae* proteomics and biomarkers – an overview. *Proteomes*, 2021, **9**, 1–18.
12. Ridley, D. S. and Jopling, W. H., Classification of leprosy according to immunity. A five-group system. *Int. J. Lepr. Other Mycobact. Dis.*, 1966, **34**, 255–273.
13. WHO, *Chemotherapy of Leprosy for Control Programmes*, World Health Organization – Technical Report, Services, 1982.
14. Arif, T., Dorjay, K., Adil, M. and Sami, M., Classification of leprosy – from past to present. *J. Pak. Assoc. Dermatol.*, 2018, **28**, 95–99.
15. Johnstone, P. A., The search for animal models of leprosy. *Int. J. Lepr. Other Mycobact. Dis.*, 1987, **55**, 535–547.
16. Shepard, C. C., The experimental disease that follows the injection of human leprosy bacilli into foot-pads of mice. *J. Exp. Med.*, 1960, **112**, 445–454.
17. Kirchheimer, W. F. and Storrs, E. E., Attempts to establish the armadillo (*Dasypus novemcinctus* Linn.) as a model for the study of leprosy. I. Report of lepromatoid leprosy in an experimentally infected armadillo. *Int. J. Lepr. Other Mycobact. Dis.*, 1971, **39**, 693–702.
18. Truman, R. W. *et al.*, The armadillo as a model for peripheral neuropathy in leprosy. *ILAR J.*, 2014, **54**, 304–314.
19. Scollard, D. M., Adams, L. B., Gillis, T. P., Krahenbuhl, J. L., Truman, R. W. and Williams, D. L., The continuing challenges of leprosy. *Clin. Microbiol. Rev.*, 2006, **19**, 338–381.
20. Vera-cabrera, L. *et al.*, *Mycobacterium leprae* infection in a wild nine banded armadillo Nuevo Leon, Mexico. *Emerg. Infect. Dis.*, 2022, **27**, 27–29.
21. Duthie, M. S. *et al.*, LepVax, a defined subunit vaccine that provides effective pre-exposure and post-exposure prophylaxis of *M. leprae* infection. *npj Vaccines*, 2018, **3**.
22. Adams, L. B., Susceptibility and resistance in leprosy: studies in the mouse model. *Immunol. Rev.*, 2021, **301**, 157–174.
23. Kim, I. K., The trend of leprosy treatment. *Korean Lepr. Bull.*, 2015, **48**, 13–15.
24. Dogra, S., Narang, T. and Kumar, B., Leprosy – evolution of the path to eradication. *Indian J. Med. Res.*, 2013, **137**, 15–35.
25. Sahoo, M. R. *et al.*, Hydnocarpus: an ethnopharmacological, phytochemical and pharmacological review. *J. Ethnopharmacol.*, 2014, **154**, 17–25.
26. Faget, G. H., Pogge, R. C., Johansen, F. A., Dinan, J. F., Prejean, B. M. and Eccles, C. G., The promin treatment of leprosy. A progress report. *Int. J. Lepr. Other Mycobact. Dis.*, 1966, **34**, 298–310.
27. Wozel, G. and Blasum, C., Dapsone in dermatology and beyond. *Arch. Dermatol. Res.*, 2014, **306**, 103–124.
28. Coleman, M. D., Dapsone: modes of action, toxicity and possible strategies for increasing patient tolerance. *Br. J. Dermatol.*, 1993, **129**, 507–513.

29. Pieters, F. A. and Zuidema, J., The absolute oral bioavailability of dapsone in dogs and humans. *Int. J. Clin. Pharmacol. Ther. Toxicol.*, 1987, **25**, 396–400.
30. Zhu, Y. I. and Stiller, M. J., Dapsone and sulfones in dermatology: overview and update. *J. Am. Acad. Dermatol.*, 2001, **45**, 420–434.
31. Zuidema, J., Hilbers-Modderman, E. S. M. and Merkus, F. W. H. M., Clinical pharmacokinetics of dapsone. *Clin. Pharmacokinet.*, 1986, **11**, 299–315.
32. Tian, W., Shen, J., Zhou, M., Yan, L. and Zhang, G., Dapsone hypersensitivity syndrome among leprosy patients in China. *Lepr. Rev.*, 2012, **83**, 370–377.
33. Venkatesan, K., Clinical pharmacokinetic considerations in the treatment of Patients with leprosy. *Clin. Pharmacokinet.*, 1989, **16**, 365–386.
34. Kar, H. K. and Gupta, R., Treatment of leprosy. *Clin. Dermatol.*, 2015, **33**, 55–65.
35. Nakata, N., Kai, M. and Makino, M., Mutation analysis of the *Mycobacterium leprae folP1* gene and dapsone resistance. *Antimicrob. Agents Chemother.*, 2011, **55**, 762–766.
36. Nisha, J., Ramanathan, K., Nawaz Khan, F., Dhanasekaran, D. and Shanthi, V., Discovery of a potential lead compound for treating leprosy with dapsone resistance mutation in *M. leprae folP1*. *Mol. Biosyst.*, 2016, **12**, 2178–2188.
37. McClure, W. R. and Cech, C. L., On the mechanism of rifampicin inhibition of RNA synthesis. *J. Biol. Chem.*, 1978, **253**, 8949–8956.
38. Acocella, G., Clinical pharmacokinetics of rifampicin. *Clin. Pharmacokinet.*, 1978, **3**, 108–127.
39. Cambau, E. *et al.*, Antimicrobial resistance in leprosy: results of the first prospective open survey conducted by a WHO surveillance network for the period 2009–15. *Clin. Microbiol. Infect.*, 2018, **24**, 1305–1310.
40. Richardus, J. H. *et al.*, Leprosy post-exposure prophylaxis with single-dose rifampicin (LPEP): an international feasibility programme. *Lancet Glob. Heal.*, 2021, **9**, e81–e90.
41. Riccardi, N. *et al.*, Clofazimine: an old drug for never-ending diseases. *Future Microbiol.*, 2020, **15**, 557–566.
42. Cholo, M. C., Steel, H. C., Fourie, P. B., Germishuizen, W. A. and Anderson, R., Clofazimine: current status and future prospects. *J. Antimicrob. Chemother.*, 2012, **67**, 290–298.
43. Singh, H., Azad, K. and Kaur, K., Clofazimine-induced enteropathy in a patient of leprosy. *Indian J. Pharmacol.*, 2013, **45**, 197–199.
44. Morrison, N. E. and Marley, G. M., Clofazimine binding studies with deoxyribonucleic acid. *Int. J. Lepr. Other Mycobact. Dis.*, 1976, **44**, 475–481.
45. Ren, Y. R. *et al.*, Clofazimine inhibits human Kv1.3 potassium channel by perturbing calcium oscillation in T lymphocytes. *PLoS ONE*, 2008, **3**, 1–11.
46. Feng, P. C., Fenselau, C. C. and Jacobson, R. R., Metabolism of clofazimine in leprosy patients. *Drug Metab. Dispos.*, 1981, **9**, 521–524.
47. Yuan, S. *et al.*, Clofazimine broadly inhibits coronaviruses including SARS-CoV-2. *Nature*, 2021, **593**, 418–423.
48. Alangaden, G. J. and Lerner, S. A., The clinical use of fluoroquinolones for the treatment of mycobacterial diseases. *Clin. Infect. Dis.*, 1997, **25**, 1213–1221.
49. Hooper, D. C. and Jacoby, G. A., Topoisomerase inhibitors: fluoroquinolone mechanisms of action and resistance. *Cold Spring Harb. Perspect. Med.*, 2016, **6**, 1–21.
50. Dalhoff, A., Pharmacodynamics of fluoroquinolones. *J. Antimicrob. Chemother.*, 1999, **43**, 51–59.
51. Venkatesan, K., Pharmacokinetics and drug interactions of newer anti-leprosy drugs. *Indian J. Dermatol. Venereol. Leprol.*, 1997, **63**, 148–152.
52. Sales-Marques, C. *et al.*, Genetic polymorphisms of the IL6 and NOD2 genes are risk factors for inflammatory reactions in leprosy. *PLoS Negl. Trop. Dis.*, 2017, **11**, 1–16.
53. Raharolahy, O. *et al.*, A case of fluoroquinolone-resistant leprosy discovered after 9 years of misdiagnosis. *Case Rep. Infect. Dis.*, 2016, **2016**, 1–4.
54. Pai, V. V., Second line anti-leprosy drugs: Indian experience. *Indian J. Drugs Dermatol.*, 2020, **6**, 1–4.
55. Narang, T., Arshdeep and Dogra, S., Minocycline in leprosy patients with recent onset clinical nerve function impairment. *Dermatol. Ther.*, 2017, **30**, 1–4.
56. Ji, B., Perani, E. G. and Grosset, J. H., Effectiveness of clarithromycin and minocycline alone and in combination against experimental *Mycobacterium leprae* infection in mice. *Antimicrob. Agents Chemother.*, 1991, **35**, 579–581.
57. Taylor, E. D. and Cahu, A., Tetracycline resistance mediated by ribosomal protection. *Antimicrob. Agents Chemother.*, 1996, **40**, 1–5.
58. Williams, D. L. and Gillis, T. P., Drug-resistant leprosy: monitoring and current status. *Lepr. Rev.*, 2012, **83**, 269–281.
59. Saivin, S. and Houin, G., Clinical pharmacokinetics of doxycycline and minocycline. *Clin. Pharmacokinet.*, 1988, **15**, 355–366.
60. Nelis, H. J. and De Leenheer, A. P., Metabolism of minocycline in humans. *Drug Metab. Dispos.*, 1982, **10**, 142–146.
61. Vester, B. and Douthwaite, S., Macrolide resistance conferred by base substitutions in 23S rRNA. *Antimicrob. Agents Chemother.*, 2001, **45**, 1–12.
62. Arikata, M. *et al.*, Efficacy of clarithromycin against H5N1 and H7N9 avian influenza a virus infection in cynomolgus monkeys. *Antiviral Res.*, 2019, **171**, 1–6.
63. Gunawan, H., Sasmojo, M., Putri, H. E., Avriyanti, E., Hindritiani, R. and Suwarsa, O., Clarithromycin efficacy in multibacillary leprosy therapy. *Int. J. Mycobacteriol.*, 2018, **7**, 152–155.
64. WHO, Chemotherapy of Leprosy, Report of a WHO Study Group, World Health Organization, Geneva, Switzerland, 1993.
65. WHO, WHO Expert Committee on Leprosy: Seventh report. World Health Organization, Geneva, Switzerland, 1998.
66. WHO, Multi-drug Therapy Against Leprosy: Development and Implementation over the past 25 years, World Health Organization, 2004.
67. Anusuya, S. and Natarajan, J., Multi-targeted therapy for leprosy: *in silico* strategy to overcome multi drug resistance and to improve therapeutic efficacy. *Infect. Genet. Evol.*, 2012, **12**, 1899–1910.
68. Manickam, P. *et al.*, International open trial of uniform multidrug therapy regimen for leprosy patients: findings and implications for national leprosy programmes. *Indian J. Med. Res.*, 2016, **144**, 525–535.
69. Tiendrebeogo, A., Andriamiandrisoa, M., Vololoarinosinjatovo, M. M., Andrianarisoa, S. H., Randriamitantsoa, J. and Bide, L., Evaluation of accompanied MDT (AMDT) in Madagascar. *Commun. Dis. Bull. African Reg.*, 2004, **2**, 5–8.
70. Malathi, M. and Thappa, D. M., Fixed-duration therapy in leprosy: limitations and opportunities. *Indian J. Dermatol.*, 2013, **58**, 93–100.
71. Bailey, M. A., Na, H., Duthie, M. S., Gillis, T. P., Lahiri, R. and Parish, T., Nitazoxanide is active against *Mycobacterium leprae*. *PLoS ONE*, 2017, **12**, 1–9.
72. Duthie, M. S., Gillis, T. P. and Reed, S. G., Advances and hurdles on the way toward a leprosy vaccine. *Hum. Vaccin.*, 2011, **7**, 1172–1183.
73. Geluk, A. *et al.*, New biomarkers with relevance to leprosy diagnosis applicable in areas hyperendemic for leprosy. *J. Immunol.*, 2012, **188**, 4782–4791.
74. Fischer, E. A. J., de Vlas, S. J., Habbema, J. D. F. and Richardus, J. H., The long term effect of current and new interventions on the new case detection of leprosy: a modeling study. *PLoS Negl. Trop. Dis.*, 2011, **5**, e1330.
75. Steinmann, P., Reed, S. G., Mirza, F., Hollingsworth, T. D. and Richardus, J. H., Innovative tools and approaches to end the transmission of *Mycobacterium leprae*. *Lancet Infect. Dis.*, 2017, **17**, e298–e305.

76. Gormus, B. J. and Meyers, W. M., Under-explored experimental topics related to integral mycobacterial vaccines for leprosy. *Expert Rev. Vaccines*, 2003, **2**, 791–804.
77. Gupte, M. D., *Indian J. Lepr.*, 1998, **70**, 369–388.
78. Sharma, P. *et al.*, Immunoprophylactic effects of the anti-leprosy Mw vaccine in household contacts of leprosy patients: clinical field trials with a follow up of 8–10 years. *Lepr. Rev.*, 2005, **76**, 127–143.
79. Singh, N. B., Lowe, A. C. R. E., Rees, R. J. W. and Colston, M. J., Vaccination of mice against *Mycobacterium leprae* infection. *Infect. Immun.*, 1989, **57**, 653–655.
80. Singh, N. B., Srivastava, A., Gupta, H. P. and Kumar, A. S. S., Induction of lepromin positivity in monkeys by a candidate antileprosy vaccine: *Mycobacterium habana*. *Int. J. Lepr. Other Mycobact. Dis.*, 1991, **59**, 317–320.
81. Wakhlu, A., Gaur, S. P., Kaushal, G. P., Misra, A., Asthana, P. and Sircar, A. R., Response of *Mycobacterium habana* vaccine in patients with lepromatous leprosy and their household contacts. A pilot clinical study. *Lepr. Rev.*, 2001, **72**, 179–191.
82. Duthie, M. S., Casper, C. and Reed, S. G., Second coming: the re-emergence and modernization of immunotherapy by vaccines as a component of leprosy control. *Future Microbiol.*, 2018, **13**, 1449–1451.
83. Craig, J., MacRae, C., Melvin, R. G. and Boggild, A. K., Case report: a case of type 1 leprosy reaction and dapsone hypersensitivity syndrome complicating the clinical course of multibacillary leprosy. *Am. J. Trop. Med. Hyg.*, 2019, **100**, 1145–1148.
84. Girling, D. J. and Hitze, K. L., Adverse reactions to rifampicin. *Bull. World Health Organ.*, 1979, **57**, 45–49.
85. Garrelts, J. C., Clofazimine: a review of its use in leprosy. *Ann. Pharmacother.*, 1991, **25**, 525–531.
86. Mathews, B., Thalody, A. A., Miraj, S. S., Kunhikatta, V., Rao, M. and Saravu, K., Adverse effects of fluoroquinolones: a retrospective cohort study in a South Indian tertiary healthcare facility. *Antibiotics*, 2019, **8**, 1–17.
87. Martins, A. M., Marto, J. M., Johnson, J. L. and Graber, E. M., A review of systemic minocycline side effects and topical minocycline as a safer alternative for treating acne and rosacea. *Antibiotics*, 2021, **10**, 1–23.
88. Şengeze, N., Yürekli, V. A., Koyuncuoğlu, H. R. and Kırbaş, S., Permanent taste and smell disorders induced by clarithromycin: a case report. *Türk. Nöroloi Derg.*, 2015, **21**, 34–36.
89. Wani, G. H. M. U. D., Lone, S. U. D., Khursheed, B., Sayeed, S. I. and Wani, H. A., Clinical evaluation of multibacillary leprosy patients after fixed duration multidrug therapy (FD-MDT) of one year: a hospital based study. *Indian J. Sci. Res.*, 2018, **4**, 603–606.
90. Ji, B. and Grosset, J., Combination of rifapentine-moxifloxacin-minocycline (PMM) for the treatment of leprosy. *Lepr. Rev.*, 2000, **71**, S81–S87.
91. Ebenezer, G. J. and Job, C. K., Histopathological activity in paucibacillary leprosy patients after ROM therapy.pdf. *Int. J. Lepr. Other Mycobact. Dis.*, 1999, **67**, 409–413.
92. Kumar, A. G. B., Multibacillary leprosy: follow up observations on 19 patients treated with 12 monthly doses of rifampicin, ofloxacin and minocycline therapy in Agra. *Indian J. Dermatol. Venereol. Leprol.*, 2014, **80**, 155–159.
93. Khang, T. H., Panikar, V., Lanh, P. H., Minh, T. T. and Hai, P. H., Treatment of leprosy with ofloxacin – containing combined drug regimens in Vietnam. *Madridge J. Dermatol. Res.*, 2019, **4**, 96–99.
94. Vivekkumar, S. P. *et al.*, Levofloxacin (L-Ofloxacin) in paucibacillary leprosy. *Glob. J. Pharmacol.*, 2010, **4**, 117–121.
95. Pardillo, F. E. F. *et al.*, Powerful bactericidal activity of moxifloxacin in human leprosy. *Antimicrob. Agents Chemother.*, 2008, **52**, 3113–3117.
96. Ji, B., Chauffour, A., Andries, K. and Jarlier, V., Bactericidal activities of R207910 and other newer antimicrobial agents against *Mycobacterium leprae* in mice. *Antimicrob. Agents Chemother.*, 2006, **50**, 1558–1560.
97. Dhople, A. M., *In vitro* activity of epiroprim, a dihydrofolate reductase inhibitor, singly and in combination with brodimoprim and dapsone, against *Mycobacterium leprae*. *Int. J. Antimicrob. Agents*, 1999, **12**, 319–323.
98. Chan, G. P. *et al.*, Clinical trial of Sparfloxacin for lepromatous leprosy. *Antimicrob. Agents Chemother.*, 1994, **38**, 61–65.
99. Franzblau, S. G. *et al.*, Clinical trial of fusidic acid for lepromatous leprosy. *Antimicrob. Agents Chemother.*, 1994, **38**, 1651–1654.
100. Gelber, R. H., The activity of amoxicillin plus clavulanic acid against *Mycobacterium leprae* in mice. *J. Infect. Dis.*, 1991, **163**, 1374–1377.
101. Ji, B. *et al.*, Antimycobacterial activities of two newer ansamycins, R-76-1 and DL 473. *Int. J. Lepr.*, 1986, **54**, 563–577.

ACKNOWLEDGEMENT. We thank DST-INSPIRE, New Delhi; Director, ICMR-NJIL and OMD, Agra, and GLA University, Mathura for the constant support and encouragement.

Received 24 January 2023; revised accepted 16 June 2023

doi: 10.18520/cs/v125/i3/253-267