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REVIEW ESSAY

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Global distribution of treatment resistance gene markers for leishmaniasis

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Abstract

Background: Pentavalent antimonials (Sb(V)) such as meglumine antimoniate (Glucantime®) and sodium stibogluconate (Pentostam®) are used as first-line treatments for leishmaniasis, either alone or in combination with second-line drugs such as amphotericin B (Amp B), miltefosine (MIL), methotrexate (MTX), or cryotherapy. Therapeutic aspects of these drugs are now challenged because of clinical resistance worldwide.

Methods: We reviewedthe recent original studies were assessed by searching in electronic databases such as Scopus, Pubmed, Embase, and Web of Science.

Results: Studies on molecular biomarkers involved in drug resistance are essential for monitoring the disease. We reviewed genes and mechanisms of resistance to leishmaniasis, and the geographical distribution of these biomarkers in each country has also been thoroughly investigated.

Conclusion: Due to the emergence of resistant genes mainly in anthroponotic Leishmania species such as L. donovani and L. tropica, as the causative agents of ACL and AVL, respectively, selection of an appropriate treatment modality is essential. Physicians should be aware of the presence of such resistance for the selection of proper treatment modalities in endemic countries.

KEYWORDS
drug resistance, gene markers, global distribution, leishmaniasis

1 | INTRODUCTION

The protozoan parasites belonging to the genus *Leishmania* are pathogenic agents of a complex and non-contagious disease, leishmaniasis.[1,2] Different clinical manifestations are present for this tropical disease ranging from benign self-healing cutaneous (CL) and mucocutaneous (MCL) to a deadly visceral (VL) leishmaniasis.[3] The major species to cause CL in the Old World consist of *Leishmania major* (*L. major*) and *Leishmania tropica* (*L. tropica*).[4] Over 70% of the global CL cases occur in Algeria, Afghanistan, Colombia, Iran, Syria, Ethiopia, North Sudan, Costa Rica, Brazil, and Peru.[5] Chemotherapy is a crucial measure to control leishmaniasis.[3] Current treatments are based on pentavalent antimonials (SbV) such as meglumine antimoniate (Glucantime®) and sodium stibogluconate (Pentostam®) as the first-line drugs alone or combined with second-choice drugs including amphotericin B (Amp B), miltefosine (MIL), methotrexate (MTX), or cryotherapy. However, the toxic adverse effects of these drugs and difficulty with distribution make these options less than ideal. Unfortunately, therapeutic aspects of these drugs are now challenged because of clinical resistance in many parts of the world.

Resistance to these drugs has become a serious problem in the treatment of leishmaniasis in some endemic areas.

Studies on molecular biomarkers involved in drug resistance are essential for monitoring the disease.[6] This phenomenon is probably an interaction between efflux, uptake, sequestration, mutation, or downregulation of an uptake system controlled by *Leishmania* genes.[7,8] The response rate to anti-*Leishmania* drugs varies between species and strains of *Leishmania*. However, this function's molecular and biochemical mechanisms are unknown.[9] The drug resistance mechanisms have often been studied in laboratory-generated strains or field-resistant strains obtained from patients in endemic regions, suggesting the involvement of different pathways. Due to the increasing rate of drug-resistant leishmaniasis cases to control the disease globally, identifying genes in each species and country is highly vital. This article aimed to review genes and mechanisms underlying resistance to leishmaniasis. All studies conducted so far have been considered in this review. Also, the spatial distribution of these biomarkers in each country has thoroughly been investigated.

2 | DRUG RESISTANCE GENE MARKERS

2.1 | Aquaglyceroporin (AQP1)

AQP1 are channel proteins that pass through the water, glycerol, and other uncharged molecules such as Sb (III) across the membranes (Figure 1). AQP1 helps the cell afford the osmotic pressure.[9] Sb (V) is a prodrug that is reduced within the human and parasite into the toxic trivalent form (Sb (III)).[10] Sb (III) enters cells by AQP1 that is energy-independent.[11] In in vitro studies, downregulation of AQP1 and high levels of trypanothione (T[SH]2)[12] have been evidenced.[13] Some studies propose that deletion of the AQP1 allele demonstrated to cause an increase in resistance to Sb (V) may be a mechanism resulting in downregulation of an uptake system.[13] Recent studies have proposed that the neutral Sb (OH)3 species serve as the substrate for AQP1 and transport within the parasite cell. The differential concentrations of Sb (V) and Sb (III) in *Leishmania* are evidence that Sb (V) uses a different way of entry.[11]

2.2 | ATP-binding cassette (ABC) transporters

Sb(III) conjugate with (T[SH]2) or glutathione(GSH), and this complex has packaged within vesicles or exited from the parasite by ABC transporters.[14] LABCI4 belongs to the ABCI subfamily, which increases the efflux of thiols and Sb(III), thereby producing resistance to antimonials in *L. major*. This transporter is in both the plasma membrane and mitochondria in *Leishmania*. LABCI4 is a pump capable of distinguishing thiol-conjugated metals.[15] The ABCC3 transporter localized in vesicular membranes near the flagellar pocket was known in trivalent arsenate (As (III)) and Sb (III) *Leishmania*-resistant isolates, and studies were proved that they offer the capability to transport thiol-conjugated metals. It has also been demonstrated that the

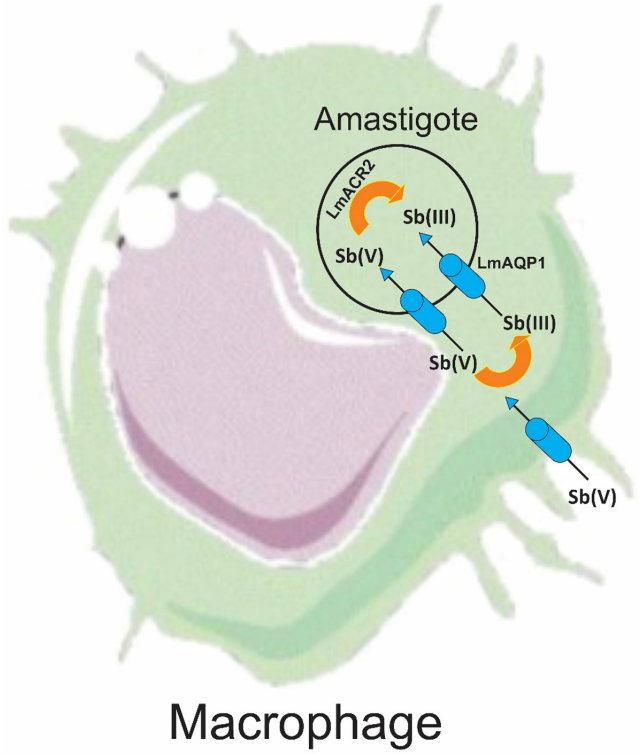

FIGURE 1 Schematic models of drug delivery in antimony resistant and sensitive *Leishmania*. AQP1, aquaglyceroporin 1; MRPA, multidrug resistance-associated protein A; EP, efflux pump; EC, the extracellular concentration of Sb; IC, the intracellular concentration of Sb; VC, the vacuolar concentration of Sb; T, rate constant for passive transport; Tma, transport rate constant by MRPA or ATe; Te, rate constant of active efflux; Ts, rate constant for secretion

MRPA-enriched vesicles possibly cooperate in a secretion pathway that reduces antimony concentration. It is also significant that either increased efflux or decreased influx of Sb (III) has been studied in *Leishmania*-resistant mutants overexpressing MRPA.[16] There was also no link between MRPA expression in the parasite and the degree of antimony intracellular concentration.[16] The other studies have shown that the overexpression of Pgp-like and MRP1-like proteins was illustrated in both of the antimony-resistant isolates of *L. donovani*, and overexpression was illustrated in both Sb (V)-resistant isolates of *L. donovani* and the plasma membrane of macrophages (MQ). This parasite effluxes the drug, reducing concentration Sb (III) in intracellular and parasite survival. On the other hand, efflux pump overexpression was not shown in antimony-sensitive *Leishmania* infected MQ.[17] This document proves the vesicle-mediated cross-talking between *Leishmania* and host cells.[18]

ABCG transporters even have been related to drug resistance.[19] LiABCG6 is located at the *Leishmania* plasma membrane. This half-transporter confers resistance to the sitamaquine and miltefosine when overexpressed by reducing intracellular drug concentration and short-chain fluorescent phospholipid analogs of phosphatidylethanolamine, phosphatidylserine, and phosphatidylcholine. As a whole, these results indicated that LiABCG6 could be implicated in

drug resistance and phospholipid trafficking.[19]LABCG4 transporter has been preoccupied with phosphatidylcholine transport and confers resistance to MIL.[20] LABCG2 is in vesicles that connect with the plasma membrane throughout exocytosis. Overexpressing the LABCG2 transporter gene in resistant isolates showed a decreased Sb (III) concentration due to increasing drug existence. Also, LABCG2 was capable of exporting thiols with Sb (III).

Leishmania LABCG2 transporter creates resistance to antimony drugs by exocytosis through flagellar pocket and packaging metal-thiol in vesicles.[21] When LABCI4 was overexpressed in *L. major* confer resistance to antimonial drugs, As(III), and metal ions Cd (II).[15] LABCI4 is localized in both plasma membranes and mitochondria of the *Leishmania* and forms dimers to efflux the thiol-conjugated metals through a thiol-X-pump.[15] MIL is used to treat these diseases effluxes through ABC transporter and P4 ATPase. P4 ATPase by the cdc50 protein transfers MIL from the outer to the inner cell and extrudes from the parasite by the ABC transporter protein, the energy-dependent mechanism. The principal genes in the *Leishmania* amplify the portions of a gene that encodes P4 ATPase and ABC transporter and participate in resistance to MIL.[22] pentamidine resistance protein 1 (PRP1) is another ABC transporter that is produced resistance to pentamidine (PTD) in *L. infantum*.[23]

P-glycoproteins (Pgps) are also the ABC transporters.[24] They extrude drugs from the parasites[25] and tumor cells,[26] thus offering a multidrug-resistant (MDR). Pgps contain two domains, the transmembrane domain (TMD) participated in medicine efflux, and a cytosolic nucleotide-binding domain (NBD) involved in hydrolysis and ATP binding.[27] Some sesquiterpenes and flavonoids are effective against *Leishmania* MDR phenotype.[28] The flavonoids join the NBD, interact with the TMD,[28] and reverse the *L. tropica* resistance phenotype.[29] Also, some sesquiterpenes efficiently defeat the *Leishmania* MDR phenotype by increasing drug accumulation.[30] Overexpression of the LtrMDR1 leads to the weakness in drug internalization and production of the resistance to MIL in *Leishmania*.[30] The data showed that *L. donovani* mitogen-activated protein kinase 1 (LdMAPK1) regulates the expression of the Pgps reversely. The reduced activity in the Pgps pump with an increase in Ld-MAPK1 expression may cause an increased concentration of antimony in the *Leishmania*, producing it more sensitive to this drug.[31] Overexpression of PgpA has been studied in resistant isolates of *L. infantum*. The transfection of this gene demonstrates antimony resistance upon amastigotes and promastigotes of *L. infantum*.[32] The recent data have shown that the expression level of the PgpA gene in resistant L. major strains was 5-fold higher than in sensitive strains. Therefore, overexpression of this gene can create resistance isolates.[33]

2.3 | Protein 14-3-3

This protein is in all eukaryotes, from mammals to plants, and more than 100 binding partners have been known so far. The targets of protein 14-3-3 are in all subcellular sections, and their functions are varied. They include biosynthetic enzymes, transcription factors, cytoskeletal proteins, apoptosis, signaling molecules, and tumor suppressors.[34] Protein 14-3-3 is capable of joining phosphorylated proteins participating in the apoptosis pathway. This protein is overexpressed in resistance *Leishmania* isolates.[35]

2.4 | Protein 299 (P299)

This gene encodes a 299 kDa polypeptide that displays no similarities to other proteins or functional motifs. Recent experiments propose that in *L. infantum* this gene is part of a 44 kbp duplicated loci on CHR29 and CHR08 chromosomes. Overexpression of this gene in *L. infantum* confers protection against Sb(III) but also against miltefosine.[36]

2.5 | Histone

Histones exist in nuclei in eukaryote cells which are alkaline proteins that pack the DNA into structural units named nucleosomes. They are the major protein of chromatin, acting as gene expression regulation.[37] Various histone genes from kinetoplastids have been identified. The sequences of the genes coding for histoneH1, H2A, H2B, H3, and H4 have been characterized in *Leishmania* species.[38] In resistant isolates of *L. donovani*, H1, H2A, and H4 were overexpressed, stating they play a role in drug resistance.[39] Overexpression of H4 was shown in *L. major* and *L. infantum* resistance antimony.[35]

2.6 | *Leishmania*-activated C kinase gene (LACK1)

This protein is very stable in *Leishmania* species and expressed in amastigote and promastigote forms.[40] These proteins took part in RNA processing, signal transduction (ST), and cell cycle regulation.[41] Recently, it has been studied that it locates in the cytosol, and the temperature variation between the insect and the mammalian host persuades it to secretion. It joins and enhancement plasminogen activation in in vivo and participates in the invasiveness of *Leishmania*.[42] The LACK is the T-cell epitope and induces the immune response and production of T-helper 1 cell; therefore, several studies have demonstrated that the LACK gene is the target for the candidate vaccine.[43,44] LACK is essential for the infectivity and viability of *Leishmania* in the MQ.[41] LACK is required to develop an incision in BALB/c mice.[45] According to the different expressions of this antigen in sensitive and resistant isolates, this gene is the primary biomarker contributing to drug resistance.[6]

2.7 | Ubiquitin

This protein is the heat shock protein with critical roles in cellular functions such as endocytosis, degradation of defective proteins, apoptosis, and DNA repair.[46] One of its critical roles in protein

decomposition by the ubiquitin-proteasome pathway, which is protecting cells from abnormal proteins.[47] Ubiquitin through the ubiquitin-proteasome pathway, ubiquitin binds to lysine residues of the target proteins, resulting in the decomposition of the ubiquitin-tagged protein via the 26S proteasome.[48] Overexpression of this gene in *L. tropica* resistant clinical isolate could decompose oxidized proteins and protect *Leishmania* from oxidative stress related to drugs.[49]

2.8 | Amino acid permease (AAP3)

Various amino acid permease has been studied in kinetoplastids.[50] It is an arginine transporter that locates in the surface membrane of the parasite.[51] Arginine is the starter of polyamine biosynthesis[52] that is transported within *Leishmania* by AAP3.[51] Ornithine results from the breakdown of arginine by the arginase enzyme that takes part in the synthesis of T(SH)2 and polyamine.[52] The T(SH)2 is a mainly reduced thiol of *Leishmania* species and had a significant role in detoxifying antimonial components.[12] Additionally, the increased T(SH)2 in antimony resistance *Leishmania* isolates has been studied.[53] It was observed that high expression of the AAP3 gene in clinical antimony-resistant isolates of *L. tropica* contributes to increasing the T(SH)2 and, as a result, detoxification of antimonial drugs.[49]

2.9 | Phosphoglycerate kinase (PGK)

Leishmania has two PGK genes: PGKB and PGKC. PGKB code the cytosolic, and PGKC codes the glycosomal isoforms of the enzyme.[54] In amastigote and promastigote stages, PGKB and PGKC transcripts and proteins are expressed at a ratio of 4:1.[55]PGK is the key enzyme of the glycolysis pathway and plays a role in ATP production.[56] Increasing glycolysis enzymes in the antimony-resistant *Leishmania* isolates proposed requiring more energy to protect from oxidative stress. Also, overexpression of PGK increases the pyruvate that extrudes peroxides and participates in decreasing oxidative stress.[57]

2.10 | Mitogen-activated protein kinase (MAPK)

MAPKs are major regulators of ST that act in parasite virulence via intracellular proliferation, stress response,[58] flagellar morphogenesis, and apoptosis.[59] Recent studies have evidenced that Sb(III) stimulates apoptosis by inducing the MAPK signaling cascade and activation of oxygen production.[60] It is overexpression in the sensitive clinical isolates and downregulated in *L. donovani* antimony-resistant isolates and proposes that MAPK1 depends on the cell death pathway, which stimulates the cell death pathway and antimonial drugs.[61] Also, compared with sensitive *L. tropica* isolates, all transcription of this gene was reduced in clinical resistant isolates.[54]

2.11 | Protein tyrosine phosphatase (PTP)

PTP is the regulator of post-translational participation in important functions in cells, such as cell death. PTPs were classified into three groups in kinetoplastids; (1) classical PTP, (2) cell division cycle 25 phosphatase, and (3) low molecular weight phosphatase.[62] PTPs have a major function in amastigote survival and virulence in the human host.[63] It has been shown that the function of the PTPs stops by the Sb (v). This inhibition is associated with activation of the MAPK pathway eventuated in apoptosis. Also, this enzyme as a virulence factor could enhance *Leishmania* survival in humans.[63] It was demonstrated that in *L. tropica* resistant clinical isolate, upregulation of this enzyme participates with downregulation of MAPK, suggesting that overexpression of PTP induces apoptosis in resistance isolates.[54]

2.12 | Pteridine reductase 1 (PTR1)

PTR1 is an NADPH-dependent reductase that contributes to the salvage of pteridines that are necessary to develop the growth of *Leishmania*.[64] PTR1 catalyzes the reduction in biopterin and folate into their active forms, tetrahydrobiopterin, and tetrahydrofolate, respectively, which act as co-factors.[65,66] Decreased pteridines in parasites lead to reduced intracellular survival.[67] Another study with *L. major* lines demonstrated that this enzyme participates in resistance parasites against MQ oxidative stress.[64] Also, as *Leishmania* is auxotrophic for pteridines, a disordering of their salvage pathway is a therapeutic strategy. The mechanisms of resistance to antimonial drugs in *L. braziliensis* and methotrexate in *L. major* and *L. infantum* have been studied.[68]

2.13 | Tryparedoxin peroxidase (TXNPx)

TXN belongs to the thioredoxin oxidoreductase superfamily and has a WCPPC motif neighbor the catalytic pocket.[69] TXNI and TXNII are two isoforms of the TXN, where TXNI is localized in the cytosol, and TXNII is localized in mitochondria. They both have a central core of 5 stranded b sheets restricted by 4 a-helices. In mammals, it performs an equal act to glutathione peroxidase. It is a member of the 2-cysteine peroxiredoxin family, and various isoforms of TXNPx have been studied, located in the mitochondria and cytosol.[70] A major role of the cytosolic TXNPx (cTXNPx) in *Leishmania* is decreasing the balance of cytosolic tryparedoxin (cTXN) made from trypanothione, unlike the other eukaryotes that apply GSH.[71] TXN and TXNPx are conserved in *Leishmania* species.[72] Their roles are defensive against oxidative stress, chemical reduction in organic hydroperoxides (ROOH), and hydrogen peroxide (H_2O_2) into alcohol and water, respectively. They also have a critical role in DNA replication, DNA biosynthesis, and ROS regulation. Mitochondrial isoform of TXN displacements electrons to the universal minicircle sequence binding protein (UMSBP), transcription factor, and a monothiol glutaredoxin by peroxidase. TXN-TXNPx pair led to a redox

state for the UMSBP and contributed to the starting of replication of kDNA.[73,74] TXN knockout studies in *L. infantum*[75] showed the necessity of this gene in these parasites' antioxidant metabolism and survival. In *L. donovani* it is identified that cTXN protein cooperates with cTXNPx to catalyze the reduction in ROOH or H_2O_2 into alcohol or water, respectively, implying its critical role under oxidative stress situations. Also, in Amp B resistant clinical isolates of *L. donovani* the cytosolic tryparedoxin level was upregulated demonstrating its role in drug resistance.[76]

2.14 | Kinetoplastid membrane (KMP11)

KMP-11 is localized in Subcellular in *Leishmania* has proposed that may be is localized to the flagella and flagellar pocket.[77,78] It was associated with the basal flagellar body, which acts in cytokinesis.[79,80] It is amphipathic, represents membrane-active properties, and increased lipid bilayer pressure.[81,82] Decreasing in KMP-11 expression changes the activity of the transporter, such as the AQP1[8] or with putative efflux systems[83] with increased function for pumping Sb(III) out of the *Leishmania* species. In various independent studies, in isolation of Sb (III) resistant *L. infantum* cell line, it is demonstrated that the reduction in this protein but the mRNA levels have not changed. These data propose that in this resistant isolate the stability of it may be agreed to result in an enhanced turnover rate of KMP 11. Change in the post-translational modifications of this protein in resistant isolates may speed up the degradation of this protein. Also, other studies have shown N-terminal acetylation[84] and arginine methylation[82] of KMP-11 that have been signified in regulating protein stability.[85] Proteomic screen data have demonstrated downregulation of its expression in Sb (III) resistant isolates in the amastigote stage. These data have marked a differentially expressed of this protein in the resistant isolate. The expression of the KMP-11 was reduced in the drug-resistant mutant.[86]

2.15 | Gamma glutamylcysteine synthase (GSH1)

Sensitivity in Leishmania to Sb (V) varies according to intrinsic cellular metabolism, intracellular thiol levels, or membrane compounds. Thiols are decreasing factors in the conversion of Sb (V) to Sb (III), which was occurring in the presence of thiols.[87] The Sb (III) mechanisms associated with its affinity toward biomolecule consisting of sulfhydryl, including proteins, enzymes, and thiols. Sb (III) conjugate with the intracellular GSH from 1:3 and trypanothione from 1:1 and formed Sb-thiol species.[88] Other proteins such as thiols, TryR, and zinc-finger protein are molecular targets of Sb (III). These molecules bind to the Sb (III) by Cys. Sb (III) disturbs the thiol metabolism by preventing TryR and stimulating the efflux of intracellular T(SH)2 and GSH and from parasite cells.[89] This function produces oxidative stresses that participate in cell death. Sb is the complex of trypanothione or GSH with Sb (III) excreted from the cell or packaging into vesicles by ATP-binding cassette (ABC) transporters.[14] Resistance isolates of *L. killicki* and *L. infantum* represented synergistic gene overexpression of GSH1 and TRPER, and in *L. infantum* overexpression of GSH1 and MRPA in resistance, isolate has been studied.[35]

2.16 | Trypanothione reductase (TryR)

TRYR maintains an intracellular reducing environment by producing the reduced trypanothione in trypanosomatids and replacing GHS in these protozoans. TRYR gene in Leishmania is vital because attempts to delete both alleles of this gene have been unsuccessful,[90] stating that this protein is necessary for *Leishmania* survival, and reduced activity of this protein is associated with reduced survival in MQ.[90] This enzyme does not exist in mammals and can be an important drug target in *Leishmania*.[91]

2.17 | Calcineurin

Calcineurin is a protein phosphatase dependent on Ca^{2+} and calmodulin and set up by calcium and contributes to various cellular functions, including apoptosis pathway and cell survival.[41] Calcineurin is a necessary enzyme in cells for many signal transduction pathways.[92] Recent studies showed the adaptation's roles under different temperature changes and salt levels.[93] Calcineurin with heat shock proteins and other molecules generates suitable virulence and thermotolerance in *L. major*.[94] Although calcineurin is involved in surviving of cells, some data proposed that under different statuses, it could play a damaging function, such as the start of the apoptosis pathway in many organisms by the specific concentration of cytosolic reactive oxygen species (ROS),[95,96] downregulation of calcineurin have a reverse effect on apoptosis in *Leishmania* species and induced apoptosis in lymphocytes.[97] The function of this enzyme is related to Ca^{2+} concentrations cytoplasm. A study showed that elevated intracellular Ca^{2+} levels in cardiac cells induced cellular apoptosis by activating some transcriptional factors and calcineurin.[98] Also, other studies demonstrated the implication of increased intracellular Ca^{2+} concentrations in parasite death.[99] Antimony components stimulate the generation of oxidative agents, for example, hydrogen peroxide (H_2O_2) or nitric oxide (NO), that have leishmanicidal effects.[100] It is documented that oxidative stress is responsible for increasing Ca^{2+} and calcineurin activation resulting in apoptosis in the *Leishmania* parasite. Recent studies proved it as a drug resistance biomarker gene in *L. infantum* that downregulation of this biomarker prevents apoptosis and increases the survival rate of *Leishmania* by Ref. [92].

2.18 | Leucine-rich repeats (LRRs)

LinJ34.0570 gene in *L. infantum* encodes a protein with 621 amino acids and contains 26 amino acid repeats enriched in leucine and a conserved cysteine. This belongs to the superfamily of LRR proteins. This protein also exists in *L. tarentolae* and *L. major* (LmjF34.0550),

respectively, with 84% and 95% homology. No putative transmembrane domains and signal peptides were identified for LinJ34.0570.[101] LRRs are a general motif and exist in various proteins, and they produce a structural framework for interactions of the proteins.[102] In *L. major*, more than 100 proteins, including promastigote surface antigen protein 2 (PSA2), have LRR repeats. *Leishmania* species that overexpressed this LRR protein were resistant to Sb (III) as axenic amastigotes and Sb (V) as intracellular parasites.[102]

2.19 | LiMT and LiRos3 transporter genes

A common characteristic in promastigotes of MIL-resistant *Leishmania* isolates is a reduced MIL concentration that is caused either by a lack in the transport of MIL by inactivation of the *L. donovani* MIL transporter (LdMT)[103] and/or by its beta-subunit LdRos3[104] or by an enhanced efflux mediated through the overexpression of ABC transporter proteins. LdMT is a P-type ATPase gene by functional survival of the MIL-resistant line. LdMT is a member of the aminophospholipid translocase subfamily and locates in the plasma membrane of the *Leishmania*.[103] These findings confirmed the prominent role of the LiMT/LiRos3 in resistance MIL *L. donovani* and *L. infantum* isolates.[105]

2.20 | ARM58 and ARM56, HSP23

ARM56, ARM58, and HSP23 are in chromosome 34 at the telomeric end. A recent study has shown that overexpression of these genes produced antimony resistance to amastigotes. The ARM58 gene produces a 58-kDa protein that has four domains in the *Leishmania*, which confers Sb (V) resistance to amastigotes and Sb (III) resistance to promastigotes.[106] For the function of this protein, the first and the second domains are essential. The third domain is significant for generation Sb (III) resistance and transmembrane.[107] Studies have shown that the HSP23 (the small 23-kDa heat shock protein) can also cause resistance to Sb (III) in vitro.[108] All three genes can generate antimony resistance to intracellular *L. donovani* amastigotes when overexpressed. ARM58 and ARM56 (ARM58rel) are secreted via exosomes.[106]

2.21 | Serine/threonine phosphatase protein (phosphatase 2C-like proteins)

Studies on trypanosomatids phosphatases are signifying essential post-translational modifications,[109] differentiation,[110] and drug resistance.[111] Serine and Threonine (Ser/Thr) residues in eukaryotes are phosphorylated in many proteins. Ser/Thr phosphatases have three families: (1) phosphoprotein phosphatases (PPPs), (2) aspartate-based phosphatases, and (3) metallo-dependent protein phosphatases (PPM).[112] Protein phosphatase 5 genes (PP5) membering of the PPP family that is distinct from other members of this family because of its N-terminal the catalytic domain domains which contain tetratricopeptide repeat (TPR) that are important in autoinhibition and protein–protein interactions.[113] The catalytic domain of PP5 is similar to the catalytic domains of PP2A, 2B/calcineurin, and protein phosphatase 1 (PP1).[114] The role of protein PP2A has been proven in the mechanism of the effect of MTX in mammalian cells.[115] Thus MTX has likely comparable mechanisms of effect and resistance in mammalian cells, and *Leishmania*,[116] the three phosphatase-related genes, emerged as biomarker resistance. LinJ.34.2310 and LinJ.34.2320 in WT *L. infantum* are phosphatase2C-like and LinJ.12.0610. LinJ.12.0610 is a serine/threonine phosphatase protein that has a conserved protein PP2A domain and two EF-hand motifs in a fused C-terminal domain[117] that may relate to the recognized role of Sb (III) as a protein phosphatase inhibitor.[118] Treatment with anticancer drugs produces ROS, which can inactivate PP2A in mammalian cells.[119] Antimonial drugs such as Sb (III) are elevated ROS in *Leishmania*,[120] overexpression of LinJ.12.0610 allows the parasite to tolerate ROS generated on exposure to Sb (III).[23]

2.22 | Iron superoxide dismutase-A

It has been proved that *Leishmania* has an antioxidant protection system for detoxifying ROS[121]and reactive nitrogen species.[122] The metalloenzyme superoxide dismutase (SOD) is a central part of the antioxidant protection system in various protozoa of various protozoa antioxidant protection systems. It eliminates other superoxide radicals by generating them into hydrogen peroxide and oxygen.[123] Cu/Mn/ZnSOD is present in eukaryotes, but FeSODs have been identified in protozoans.[123] FeSOD is absent in humans and can be a good target for the treatment of leishmaniasis.[73] FeSOD-A and FeSOD-B are the FeSOD species, demonstrated in *L. infantum/chagasi*, *L. donovani*, and *L. tropica*.[124] Recent studies showed the high activity of superoxide dismutase in Sb (III) resistant *L. infantum* and *L. braziliensis* in in vitro conditions and *L. donovani* in clinical isolates.[125]

2.23 | Folate transporter 1 (FT1)

The pathway of folate biosynthesis is used to make many medicines. Folates are made of a pterin that combines glutamic acid and para-aminobenzoic acid. Resistance to MTX generated by various genes also decreased the concentration of the drug in the *Leishmania*.[126] Studies have shown that the reduction in MTX in *Leishmania* also reduces folate uptake proposing that the expression of a joint folate/MTX transporter is highly downregulated in MTX-resistant isolates of *Leishmania*. Folate transport regulates the growth stage of the *Leishmania* in both the logarithmic and stationary phases. Recent studies presented that folate transporter 1 (FT1) is a member of the BT1 family responsible for the affinity of folate and MTX transporter in *Leishmania*. Variation of the expression of this gene-modified antifolate sensitivity. This protein was localized in the plasma membrane.[127] Recent data showed that an FT1 disrupted in *L. infantum*

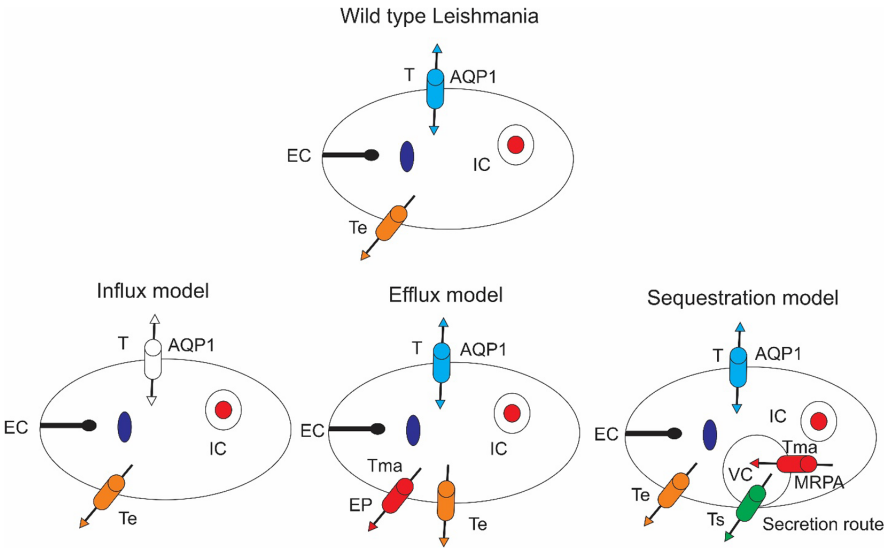

FIGURE 2 Model of Pentostam action in MQ and amastigotes of *Leishmania*

MTX-resistant mutant corresponds to the leading folate transporter in the parasite. It was proved as the major folate transporter through gene targeting studies. Variation of the FT1 gene expression changed the sensitivity of *L. infantum* to the MTX.[128]

2.24 | HSP83

The *Leishmania* HSP83 is similar to the mammalian HSP90. HSP90 was recognized to be an inverted controller of the mitochondria-dependent apoptosis pathway.[129] HSP90 is associated with Bcl-2 and inhibited mitochondrial apoptotic cascades.[130] The collaboration of HSP90 in programmed cell death (PCD) confirms the role of HSP83 in drug-induced PCD in *Leishmania*. HSP83 interacts with other proteins in *Leishmania* to reversely regulate the mitochondrial apoptotic pathway.[131] In antimony resistance, clinical isolates of *L. donovani* were shown the overexpression of HSP83, and its role was proved in antimony resistance by gene targeting in sensitive *L. donovani* parasite.[132] In recent study demonstrated that the overexpression of this gene was elevated in four out of the ten resistant isolates.

Also, there was a slight correlation between the antimony susceptibility and HSP83 gene expression, demonstrating that this gene is not the only cause for resistance in clinical isolates. The resistant clinical isolate presented resistance to other medicines, including MIL and Amp B. proteomic studies have demonstrated various proteins differentially expressed, proposing that PCD is changed in the resistant isolates. Actually, drug-induced PCD has changed the markers of apoptosis in the Sb (V) resistant isolate. The HSP83 and the SKCRP14.1 demonstrated two proteins to be involved in the drug-induced PCD. HSP83 enhanced resistance and decreased drug-mediated PCD by intervention with the mitochondrial membrane potential, also SKCRP14.1 initiated PCD but protected against MIL-induced PCD. This finding demonstrated the role of PCD in drug sensitivity or resistance in the *Leishmania* species.[131]

2.25 | Small kinetoplastid calpain-related protein (SKCRP14.1)

This protein belongs to the family of calcium-dependent cysteine proteases.[133] This new protein was downregulated within the clinical isolate of *L. donovani* from India, and overexpression of this gene in the parasite resensitized the parasite to antimonial drugs through induced PCD. SKCRP14.1 overexpression in the existence of Sb (III) only quantitatively.[131] High expression of SKCRP14.1 increased the antimonial susceptibility in *L. donovani* but, interestingly, caused an increased resistance to MIL. Considering these resistance phenotypes, high expression of SKCRP14.1 caused increased protection versus MIL-induced PCD. Therefore, a change in SKCRP14.1 expression had contradictory effects on sensitivity to antimonials and MIL.[131] SKCRP14.1 and HSP83 were demonstrated to be closely related to the drug-induced PCD phenotype. SKCRP14.1 elevated antimonial-induced PCD but protected clearly into MIL-induced PCD, whiles HSP83 increased the drug resistance and reduced drug-induced PCD activation via participating with the mitochondrial membrane potential.[131]

2.26 | LmACR2

Sb (V) must be diminished to Sb (III) to make this medication dynamic. MQ catches Sb (V), and a portion of it is reduced to Sb (III), which is then transported into the amastigotes by AQP1. The other bit of this medication decreased to Sb (III) by LmACR2 and TDR1.[134] The two pathways of drug activity would be related to the expression of their relevant components in both MQ and *Leishmania*. This had been varied in different species of *Leishmania*. LmACR2, in *L. major*, is the first known as metalloid reductase with a physiological function in activating the drug.[134] Transfection of the LmACR2 gene in *L. infantum* enhanced the susceptibility to Pentostam in intracellular amastigotes (Figure 2). These findings

suggest that this gene is responsible for reducing the pentavalent antimonial compounds in pentostam® to the active Sb (III) in *Leishmania*.[134]

2.27 | Thiol-dependent reductase I (TDR1)

This protein is an enzyme detected in *Leishmania* species involved in deglutathionylation and activation of Sb (V) used in the treatment of leishmaniosis.[135] In *Leishmania* spp, TDR1 is involved in redox regulation and promoting sensitivity to the antimonial prodrugs glucantime and pentostam, known as the first-line treatment of Leishmaniosis.[136] The therapeutic function of these drugs is to decrease the pentavalent species to trivalent species that are toxic. This procedure happens gradually under in vitro conditions within sight of glutathione (GSH) or the T(SH)2, particularly at low pH as found in the parasitophorous vacuole in which Leishmania dwells intracellularly in MQs.[137] TDR1, within sight of GSH, catalyzes the decrease of Sb (V) in vitro condition and thus could actuate the antimonial prodrugs.[138] This enzyme is more abundant in amastigote form and the amastigotes are intensely more sensitive to Sb (V) than promastigotes.[139] This enzyme is a member of the glutathione-S-transferase (GST) superfamily[138] which involves biological events such as signaling processes, stress response, and xenobiotic detoxification.[140]

2.28 | Heat shock protein70 (hsp70)

Conserved proteins of this class are molecular chaperones playing the leading role in maintaining cellular homeostasis approximately in all known organisms. *T. cruzi*, *T. brucei*, and *L. major* known as the Tritryps are human parasites. These parasites change their morphology in the life cycle of humans and insects. Hsp70s make these changes in different hosts and conditions also remaining viable and infective.[141] The hsp70 is part of a cellular network, which is frequently involved in protein folding processes and molecular chaperoning.[141] In *Leishmania* antimony-resistant isolates, Hsp70 has been detected to be upregulated at mRNA,[142] and protein[143] levels; this does not directly produce resistance, but it enhances the metal tolerance in the *Leishmania*. So it allows the cell to create resistance mechanisms.[142] Mutation in the hsp70 gene of *L. braziliensis* could modulate the failure of antimonial treatment in patients.[144]

3 | GEOGRAPHICAL DISTRIBUTION

Table 1 provides information on studies on resistance biomarkers gene in *Leishmania* species, based on a study including laboratory studies and clinical-resistant isolates.

In Figures 3 and 4, the geographic distribution of biomarkers is shown separately according to the study types, which are fully explained in the discussion section. These maps are drawn by ArcGIS v 10.1 software.

TABLE 1 Drug resistance gene markers in leishmaniasis

Gene	*Leishmania* species	Type of isolate	Country (reference)
AQP1	*L. major*	CRI	Iran[145]
	L. tropica	CRI	Iran[54]
	L. panamensis	LRM	USA[9]
	L. infantum	CRI	Tunisia, Algeria[35]
	L. donovani	CRI	India[146]
	L. guyanensis	LRM	Brazil[147]
	L. braziliensis	LRM	Brazil[148]
ABCI4	*L. major*	LRM	Spain[15]
ABCC3	*L. infantum*	LRM	Canada[149]
	L. donovani	CRI	India[17]
ABCG	*L. donovani*	LRM	India[150]
	L. infantum	LRM	Spain[19]
	L. major	LRM	Spain[21]
Pgps	*L. tropica*	LRM	Spain[29]
	L. donovani	CRI	India[146]
Pgp A	*L. infantum*	LRM	Canada[151]
	L. major	LRM	Iran[33]
	L. guyanensis	LRM	Brazil[152]
Protein 14-3-3	*L. major*	CRI	Algeria[35]
	L. infantum	CRI	Algeria[35]
P299	*L. major*	CRI	Algeria[35]
	L. infantum	CRI	Algeria[35]
Histon 4	*L. major*	CRI	Algeria, Tunisia[35]
	L. infantum	CRI	Algeria, Tunisia[35]
	L. donovani	CRI	India[39]
Histon H2A	*L. donovani*	CRI	India[153]
Histon H1	*L. donovani*	CRI	India[39]
LACK1	*L. tropica*	CRI	Iran[6]
Ubiquitin	*L. tropica*	CRI	Iran[49]
AAP3	*L. tropica*	CRI	Iran[49]
	L. donovani	LRM	Spain[166]
PGK	*L. tropica*	CRI	Iran[54]
MAPK	*L. tropica*	CRI	Iran[54]
	L. donovani	CRI	India[61]
PTP	*L. tropica*	CRI	Iran[54]
PTR1	*L. major*	LRM	Canada[154]
	L. braziliensis	LRM	Brazil[68]
	L. infantum	LRM	Canada[116]
TXNPx	*L. infantum*	CRI	Algeria, Tunisia[35]
	L. donovani	CRI	India[76]
	L. braziliensis	LRM	Brazil[155]
	L. major	LRM	United Kingdom[156]
KMP11	*L. infantum*	CRI	France[35]
Hsp70	*L. braziliensis*	CRI	Brazil[144]
	L. donovani	CRI	India[157]
	L. infantum	LRM	Brazil[158]

TABLE 1 (Continued)

Gene	*Leishmania* species	Type of isolate	Country (reference)
GSH1	*L. guyanensis*	CRI	Brazil[159]
	L. infantum	CRI	Tunisia[35]
	L. donovani	CRI	United Kingdom[160]
TryR	*L. major*	LRM	Canada[91]
	L. donovani	CRI	India[161]
Calcineurin	*L. infantum*	CRI	Iran[92]
LRRs	*L. infantum*	LRM	Canada[102]
	L. donovani	CRI	India[162]
LiMT and LiRos3	*L. infantum*	CRI	France[105]
	L. donovani	LRM	Spain[103]
ARM58, ARM56, and HSP23	*L. infantum*	LRM	Germany[106]
	L. donovani	LRM	Germany[106]
	L. braziliensis	CRI	Peru[163]
Serine/threonine phosphatase protein	*L. infantum*	LRM	Canada[23]
Iron superoxide dismutase A	*L. infantum*	LRM	Brazil[125]
	L. braziliensis	LRM	Brazil[125]
	L. donovani	CRI	India[164]
Folate transporter 1	*L. infantum*	LRM	Canada[128]
	L. major	LRM	Canada[165]
HSP83	*L. donovani*	CRI	India[132]
	L. infantum	LRM	Brazil[158]
	L. braziliensis	LRM	Brazil[158]
SKCRP14.1	*L. donovani*	CRI	India[131]
LmACR2	*L. infantum*	LRM	Canada[134]
	L. donovani	LRM	Canada[134]
TDR1	*L. major*	LRM	United Kingdom[138]

Abbreviations: CRI, clinical-resistant isolate; LRM, laboratory-resistant mutant.

4 | DISCUSSION

There are numerous studies about the molecular biomarkers of drug resistance in leishmaniasis. In this article, we have tried to collect these biomarkers. Several of these investigations used resistant isolates taken from patients, while others used isolates resistant to drug treatment in the laboratory. In addition, in this article, we have determined that these biomarkers have been reported in patients in various countries.

The downregulation of the AQP1 gene has been reported from resistant isolates such as *L. major* in Iran,[145] *L. tropica* in Iran,[54] *L. infantum* in Algeria, and Tunisia,[35] *L. donovani* in India[146] from patients. However, this gene has been considered a resistance marker only in laboratory conditions in *L. panamensis* (USA),[9] *L. guyanensis* (Brazil),[147] *L. braziliensis* (Brazil).[148]

The ABCI4 gene was studied in *L. major* and introduced as a drug resistance biomarker.[15]

ABCC3 gene has been investigated in resistant isolates of human specimens and is known as a resistance biomarker in *L. donovani* in India.[17] The resistance of this gene has been reported in a laboratory model in *L. infantum* in Canada.[149]

The potential for resistance to the ABCG gene has been studied only in vitro. In *L. donovani* in India[150] and *L. major*[21] and *L. infantum* in Spain[19] have been examined.

According to studies conducted in laboratory conditions in Spain, the Pgps gene can regulate drug accumulation and reverse the resistance phenotype of *L. tropica*.[29] In India, a study on resistant specimens of *L. donovani* showed this gene as a molecular marker of resistance.[146]

All research on the PgpA gene has been conducted in laboratory conditions. These researches were carried out on *L. infantum*, *L. major*, and *L. guyanensis* in Canada,[151] Iran,[33] and Brazil,[152] respectively.

Changes in the expression of the protein of 14–3-3, P299 genes have been recorded on samples taken from patients in Algeria. These resistance genes have been observed in *L. major* and *L. infantum* in this country.[35]

Research on Histon 4, Histon H2A, and Histon H1 genes showed that they were used as resistance markers in human resistance specimens. Histon 4 in Algeria and Tunisia has been studied on *L. major* and *L. infantum*[35] and in India on *L. donovani*.[39] Histon H2A,[153] Histon H1[39] genes have been investigated only in *L. donovani* in India.

The information obtained from four genes (LACK1, Ubiquitin, AAP3and PGK) have been obtained from studies conducted on patients who were resistant to treatment in Iran. These resistant isolates were *L. tropica*.[6,49] One study was done on the AAP3 gene in in vitro conditions in Spain.

Change in expression of the MAPK gene that leads to resistance to treatment has been observed in patients in Iran and India. Resistant strains of *L. tropica* and *L. donovani* have been reported in Iran and India, respectively.[54,61]

The PTP gene as a biomarker of resistance is isolated from patients only in Iran. These isolates are related to *L. tropica*.[54]

All studies on the PTR1 gene have been performed on drug resistance in *Leishmania* species in vitro conditions. These studies have been conducted on *L. major*[154] and *L. infantum*[116] and *L. braziliensis*[68] in Canada and Brazil, respectively.

Studies were conducted on the TXNPx gene in resistant strains isolated from patients in Algiers and Tunisia[35] on *L. infantum* in India and *L. donovani*.[76] Laboratory studies have been conducted on *L. major* and *L. braziliansis* in Brazil[155] and the United Kingdom, respectively.[156] Research on the KMP11 gene has been conducted as a biomarker of resistance only in a patient from France.[35]

Studies have been performed on the Hsp70 gene in human specimens on *L. braziliansis*[144] and *L. donovani* in Brazil and India,[157] respectively. A study on *L. infantum* in Brazil showed that drug resistance had been established due to this gene in vitro model.[158]

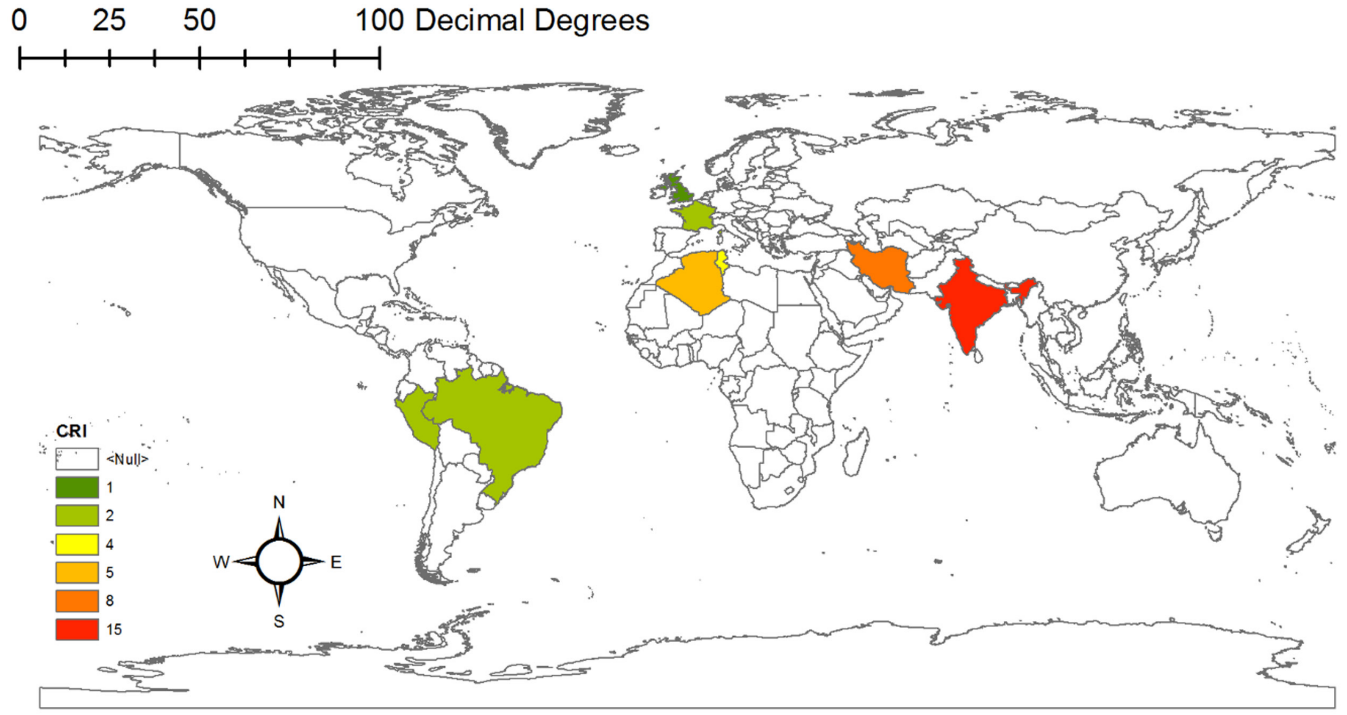

FIGURE 3 Geographical distribution of resistant biomarkers gene based on studies carried out on clinical-resistant isolate

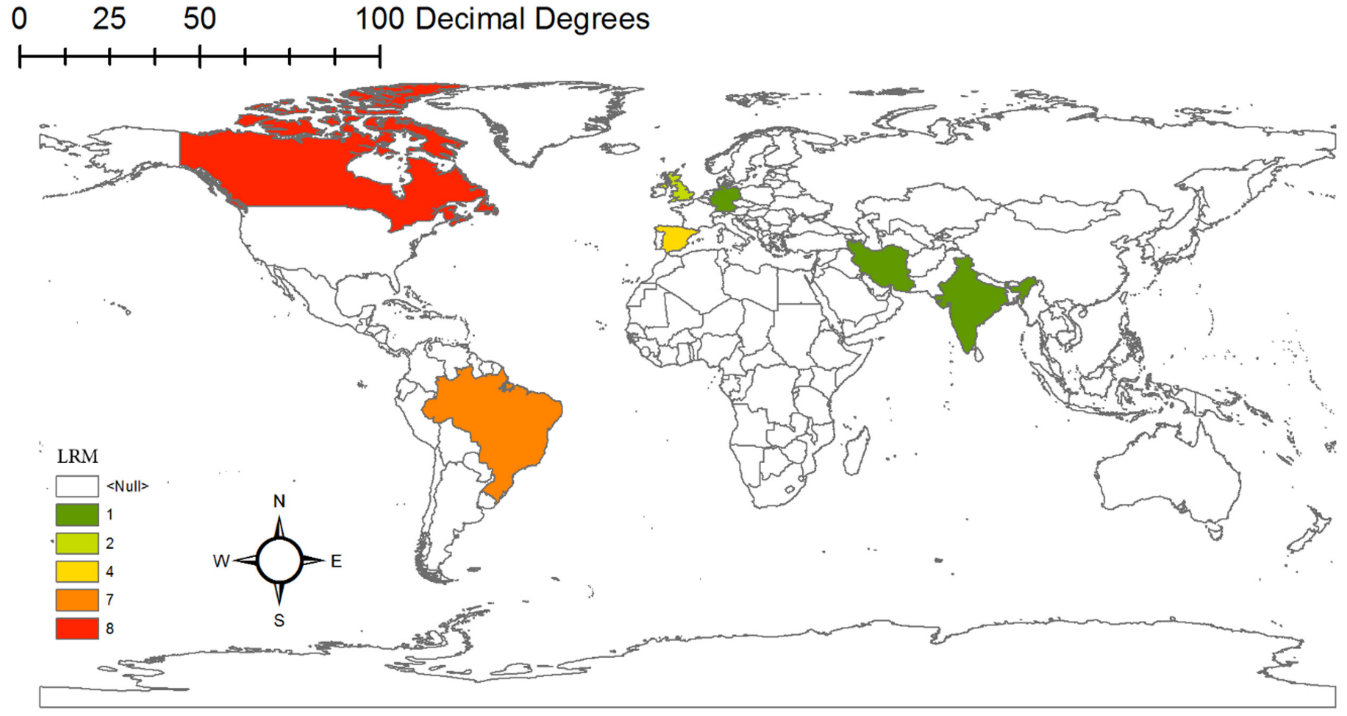

FIGURE 4 Geographical distribution of resistant biomarkers gene based on studies carried out on laboratory-resistant mutant

There are three types of research on the GSH1 gene in resistant human isolates. The studies were performed on *L. guyanensis*, *L. infantum* and *L. donovani* in Brazil,[159] Tunisia,[35] and the United Kingdom,[160] respectively.

Two investigations have been published on TryR gene. A study was carried out on *L. donovani* on a drug-resistant specimen from a patient in India.[161] Another study was conducted on *L. major* in Canada in in vitro condition.[91]

Only a study on Calcineurin gene has been published on *L. infantum* of the human-resistant specimen in Iran.[92]

The study of the LRRs gene was carried out on *L. donovani* in resistant specimens from India[162] and on *L. infantum* in Canada in a laboratory model.[102]

For LiMT and LiRos3 genes, two studies have been performed on *L. infantum* and *L. donovani*. The study has been done on these genes in *L. infantum* in human-resistant samples in France.[105] Another study reported on *L. donovani* in in vitro in Spain.[103]

Studies on ARM58, ARM56, and HSP23 genes have been conducted on *L. infantum* and *L. donovani* in in vitro conditions in Germany.[106] A survey of *L. braziliansis* has been published on resistant human specimens from Peru.[163]

In the serine/threonine phosphatase gene, only one study was done in Canada in vitro.[23]

Information about the iron superoxide dismutase A gene is available on *L. infantum* and *L. braziliensis* base laboratory studies,[125] which have been registered in Brazil, and a study on *L. donovani* from the clinical sample in India.[164]

Available information on the folate transporter 1 gene is based on the experimental study on *L. infantum*[128] and *L. major*[165] from Canada.

HSP83 gene has been investigated as a biomarker of resistance in human specimens on *L. donovani* in India.[132] In Brazil, research has been done on *L. infantum* and *L. braziliensis* in laboratory conditions.[158]

Information about the SKCRP14.1 gene has been obtained from a study conducted on human-resistant samples of *L. donovani*[131] in India.

A study on the LmACR2 gene has been carried out on *L. infantum* and *L. donovani* in laboratory conditions in Canada.[134]

The ability to create drug resistance by TDR1 gene in the laboratory has been studied on *L. major* in the United Kingdom.[138]

5 | CONCLUSION

According to current research, biomarkers of drug resistance are consistent with each country and the studied species are as follows: in Iran: AQP1 (*L. major*, *L. tropica*), LACK1 (*L. tropica*), ubiquitin (*L. tropica*), AAP3 (*L. tropica*), PGK (*L. tropica*), MAPK (*L. tropica*), PTP (*L. tropica*), Calcineurin (*L. infantum*), in Tunisia: AQP1 (*L. infantum*), Histon 4 (*L. major*, *L. infantum*), TRPER (*L. infantum*), GSH1 (*L. infantum*); in Algeria: AQP1 (*L. infantum*), Protein 14–3-3 (*L. major*, *L. infantum*), P299 (*L. major*, *L. infantum*), Histon 4 (*L. major*, *L. infantum*), TRPER (*L. infantum*), in India: AQP1 (*L. donovani*), ABCC3 (*L. donovani*), Pgps (*L. donovani*), Histon 4 (*L. donovani*), Histon H2A, Histon H1 (*L. donovani*), MAPK (*L. donovani*), TRPER (*L. donovani*), Hsp70 (*L. donovani*), Hsp70 (*L. donovani*), TryR (*L. donovani*), LRRs (*L. donovani*), Iron superoxide dismutase A (*L. donovani*), HSP83 (*L. donovani*), SKCRP14.1 (*L. donovani*), in France: KMP11 (*L. infantum*), LiMT and LiRos3 (*L. infantum*), in Brazil: Hsp70 (*L. braziliensis*), GSH1 (*L. guyanensis*), in the UK: GSH1(*L. donovani*), in Peru: ARM58, ARM56, and HSP23 (*L. braziliensis*).

The significance of these findings is determined in two areas: (1) biomarkers should be considered in the diagnosis of resistant species in each country or region. (2) What biomarkers should be studied in drug studies in each country to find and use an appropriate drug to treat resistant species?

RECOMMENDATIONS

1. Due to the emergence of resistant genes mainly in anthroponotic *Leishmania* species such as *L. donovani* and *L. tropica*, as the causative agents of ACL and AVL, respectively, selection of an appropriate treatment modality is essential.
2. The control of leishmaniasis is complicated as there is no efficacious vaccine available. Controlling vectors and reservoir hosts is practical because of numerous species implicated in the life cycle.
3. At present, combination therapy would be a proper choice for selecting a treatment modality, more importantly among the first-line agents along with the second-choice drugs or cryotherapy. The use of two anti-leishmanial simultaneously, especially when the drugs have different mechanisms of action, has the development of resistance to either of the components.
4. Physicians should be aware of the presence of such resistance for the selection of proper treatment modalities in endemic countries.

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CONFLICT OF INTEREST

The authors confirm that this article's content has no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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