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 reviewed the 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 anthropotic 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 (Sb(V)) 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. This phenomenon is probably an interaction between efflux, uptake, sequestration, mutation, or downregulation of an uptake system controlled by *Leishmania* genes. The response rate to anti-*Leishmania* drugs varies between species and strains of *Leishmania*. However, this function’s molecular and biochemical mechanisms are unknown. 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. Sb (V) is a prodrug that is reduced within the human and parasite into the toxic trivalent form (Sb (III)). Sb (III) enters cells by AQP1 that is energy-independent. In in vitro studies, downregulation of AQP1 and high levels of trypanothione (T[SH]2) have been evidenced. 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. 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.

### 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. 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. The ABC23 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 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. There was also no link between MRPA expression in the parasite and the degree of antimony intracellular concentration. 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. This document proves the vesicle-mediated cross-talking between *Leishmania* and host cells.

ABC transporters even have been related to drug resistance. 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 phosphatidyl-
lethanolamine, phosphatidylserine, and phosphatidylcholine. As a whole, these results indicated that LiABCG6 could be implicated in...
drug resistance and phospholipid trafficking. LABCG4 transporter has been preoccupied with phosphatidylcholine transport and confers resistance to MIL. 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. When LABCI4 was overexpressed in L. major confer resistance to antimonial drugs, As(III), and metal ions Cd (II). 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. 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. pentamidine resistance protein 1 (PRP1) is another ABC transporter that is produced resistance to pentamidine (PTD) in L. infantum.

P-glycoproteins (Pgps) are also the ABC transporters. They extrude drugs from the parasites and tumor cells, 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. Some sesquiterpenes and flavonoids are effective against Leishmania MDR phenotype. The flavonoids join the NBD, interact with the TMD, and reverse the L. tropica resistance phenotype. Also, some sesquiterpenes efficiently defeat the Leishmania MDR phenotype by increasing drug accumulation. Overexpression of the LtrMDR1 leads to the weakness in drug internalization and production of the resistance to MIL in Leishmania. 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. 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. 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.

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. Protein 14-3-3 is capable of joining phosphorylated proteins participating in the apoptosis pathway. This protein is overexpressed in resistance Leishmania isolates.

2.4 | Protein 299 (P299)

This gene encodes a 299kDa 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.

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. Various histone genes from kinetoplastids have been identified. The sequences of the genes coding for histone H1, H2A, H2B, H3, and H4 have been characterized in Leishmania species. In resistant isolates of L. donovani, H1, H2A, and H4 were overexpressed, stating they play a role in drug resistance. Overexpression of H4 was shown in L. major and L. infantum resistance antimony.

2.6 | Leishmania-activated C kinase gene (LACK1)

This protein is very stable in Leishmania species and expressed in amastigote and promastigote forms. These proteins took part in RNA processing, signal transduction (ST), and cell cycle regulation. 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 vivo and participates in the invasiveness of Leishmania. 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. LACK is essential for the infectivity and viability of Leishmania in the MQ. LACK is required to develop an incision in BALB/c mice. According to the different expressions of this antigen in sensitive and resistant isolates, this gene is the primary biomarker contributing to drug resistance.

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. One of its critical roles in protein...
decomposition by the ubiquitin-proteasome pathway, which is protecting cells from abnormal proteins. 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. Overexpression of this gene in L. tropica resistant clinical isolate could decompose oxidized proteins and protect Leishmania from oxidative stress related to drugs.

2.8 | Amino acid permease (AAP3)

Various amino acid permease has been studied in kinetoplastids. It is an arginine transporter that locates in the surface membrane of the parasite. Arginine is the starter of polyamine biosynthesis that is transported within Leishmania by AAP3. Ornithine results from the breakdown of arginine by the arginase enzyme that takes part in the synthesis of T(SH)2 and polyamine. The T(SH)2 is a mainly reduced thiol of Leishmania species and had a significant role in detoxifying antimonial components. Additionally, the increased T(SH)2 in antimony resistance Leishmania isolates has been studied. 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.

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. In amastigote and promastigote stages, PGKB and PGKC transcripts and proteins are expressed at a ratio of 4:1. PGK is the key enzyme of the glycolysis pathway and plays a role in ATP production. 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.

2.10 | Mitogen-activated protein kinase (MAPK)

MAPKs are major regulators of ST that act in parasite virulence via intracellular proliferation, stress response, flagellar morphogenesis, and apoptosis. Recent studies have evidenced that Sb(III) stimulates apoptosis by inducing the MAPK signaling cascade and activation of oxygen production. 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. Also, compared with sensitive L. tropica isolates, all transcription of this gene was reduced in clinical resistant isolates.

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. PTPs have a major function in amastigote survival and virulence in the human host. 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. 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.

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. PTR1 catalyzes the reduction in biopterin and folate into their active forms, tetrahydrobiopterin, and tetrahydrofolate, respectively, which act as co-factors. Decreased pteridines in parasites lead to reduced intracellular survival. Another study with L. major lines demonstrated that this enzyme participates in resistance parasites against MQ oxidative stress. 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.

2.13 | Tryparedoxin peroxidase (TXNPx)

TXN belongs to the thioredoxin oxidoreductase superfamily and has a WCPCP motif neighbor the catalytic pocket. 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. 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. TXN and TXNPx are conserved in Leishmania species. Their roles are defensive against oxidative stress, chemical reduction in organic hydroperoxides (ROOH), and hydrogen peroxide (H2O2) 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. TXN knockout studies in L. infantum showed the necessity of this gene in these parasites' antioxidant metabolism and survival. In L. donovani it is identified that cTXN protein cooperates with cTXNXp to catalyze the reduction in ROOH or H2O2 into alcohol or water, respectively, implying its critical role under oxidative stress situations. Also, in Amp B resistant clinical isolates of L. donovani the cystolic tryparedoxin level was upregulated demonstrating its role in drug resistance.

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. It was associated with the basal flagellar body, which acts in cytokinesis. It is amphipathic, represents membrane-active properties, and increased lipid bilayer pressure. Decreasing in KMP-11 expression changes the activity of the transporter, such as the AQP1 or with putative efflux systems 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 and arginine methylation of KMP-11 that have been signified in regulating protein stability. 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.

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. 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. 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 TSH2 and GSH and from parasite cells. 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. 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.

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, stating that this protein is necessary for Leishmania survival, and reduced activity of this protein is associated with reduced survival in MQ. This enzyme does not exist in mammals and can be an important drug target in Leishmania.

2.17 | Calcineurin

Calcineurin is a protein phosphatase dependent on Ca2+ and calmodulin and set up by calcium and contributes to various cellular functions, including apoptosis pathway and cell survival. Calcineurin is a necessary enzyme in cells for many signal transduction pathways. Recent studies showed the adaptation’s roles under different temperature changes and salt levels. Calcineurin with heat shock proteins and other molecules generates suitable virulence and thermotolerance in L. major. 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), downregulation of calcineurin have a reverse effect on apoptosis in Leishmania species and induced apoptosis in lymphocytes. The function of this enzyme is related to Ca2+ concentrations cytoplasm. A study showed that elevated intracellular Ca2+ levels in cardiac cells induced cellular apoptosis by activating some transcriptional factors and calcineurin. Also, other studies demonstrated the implication of increased intracellular Ca2+ concentrations in parasite death. Antimony components stimulate the generation of oxidative agents, for example, hydrogen peroxide (H2O2) or nitric oxide (NO), that have leishmanicidal effects. It is documented that oxidative stress is responsible for increasing Ca2+ 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)

Lin34.0570 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. LRRs are a general motif and exist in various proteins, and they produce a structural framework for interactions of the proteins. 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.

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) and/or by its beta-subunit LdRos3 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. These findings confirmed the prominent role of the LiMT/LiRos3 in resistance MIL L. donovani and L. infantum isolates.

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. 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. Studies have shown that the HSP23 (the small 23-kDa heat shock protein) can also cause resistance to Sb (III) in vitro. All three genes can generate antimony resistance to intracellular L. donovani amastigotes when overexpressed. ARM58 and ARM56 (ARM58rel) are secreted via exosomes.

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

Studies on trypanosomatids phosphatases are signifying essential post-translational modifications, differentiation, and drug resistance. 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). Protein phosphatase 5 genes (PP5) members of the PPP family that is distinct from other members of this family because of its N-terminal the catalytic domain domains which contain tetraatomic peptide repeat (TPR) that are important in autoinhibition and protein–protein interactions. The catalytic domain of PP5 is similar to the catalytic domains of PP2A, 2B/calcineurin, and protein phosphatase 1 (PP1). The role of protein PP2A has been proven in the mechanism of the effect of MTX in mammalian cells. Thus MTX has likely comparable mechanisms of effect and resistance in mammalian cells, and Leishmania, 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 that may relate to the recognized role of Sb (III) as a protein phosphatase inhibitor. Treatment with anticancer drugs produces ROS, which can inactivate PP2A in mammalian cells. Antimonal drugs such as Sb (III) are elevated ROS in Leishmania, overexpression of LinJ.12.0610 allows the parasite to tolerate ROS generated on exposure to Sb (III).

2.22 | Iron superoxide dismutase-A

It has been proved that Leishmania has an antioxidant protection system for detoxifying ROS and reactive nitrogen species. 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. Cu/Mn/ZnSOD is present in eukaryotes, but FeSODs have been identified in protozoans. FeSOD is absent in humans and can be a good target for the treatment of leishmaniasis. FeSOD-A and FeSOD-B are the FeSOD species, demonstrated in L. infantum/chagasi, L. donovani, and L. tropica. 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.

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. 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. Recent data showed that an FT1 disrupted in L. infantum
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 reverse 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, whereas 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 antimonials 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,130 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.142 In *Leishmania* antimony-resistant isolates, Hsp70 has been detected to be upregulated at mRNA,142 and protein143 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             | Iran145             |
|          | *L. tropica*         | CRI             | Iran54              |
|          | *L. panamensis*      | LRM             | USA9                |
|          | *L. infantum*        | CRI             | Tunisia, Algeria25  |
|          | *L. donovani*        | CRI             | India166            |
|          | *L. guyanensis*      | LRM             | Brazil147           |
|          | *L. braziliensis*    | LRM             | Brazil148           |
| ABCI4    | *L. major*           | LRM             | Spain15             |
| ABCCC3   | *L. infantum*        | LRM             | Canada149           |
|          | *L. donovani*        | CRI             | India147            |
| ABCG     | *L. donovani*        | LRM             | India160            |
|          | *L. infantum*        | LRM             | Spain19             |
|          | *L. major*           | LRM             | Spain21             |
| Pgps     | *L. tropica*         | LRM             | Spain9              |
|          | *L. donovani*        | CRI             | Iran146             |
| Pgp A    | *L. infantum*        | LRM             | Canada151           |
|          | *L. major*           | LRM             | Iran33              |
|          | *L. guyanensis*      | LRM             | Brazil152           |
| Protein 14-3-3 | *L. major*   | CRI             | Algeria35            |
|          | *L. infantum*        | CRI             | Algeria35            |
| P299     | *L. major*           | CRI             | Algeria35            |
|          | *L. infantum*        | CRI             | Algeria35            |
| Histon 4 | *L. donovani*        | CRI             | Algeria, Tunisia35   |
|          | *L. infantum*        | CRI             | Algeria, Tunisia35   |
|          | *L. donovani*        | CRI             | India39             |
| Histon H2A| *L. donovani*     | CRI             | India153            |
| Histon H1| *L. donovani*        | CRI             | India39             |
| LACK1    | *L. tropica*         | CRI             | Iran6               |
| Ubiquitin| *L. tropica*         | CRI             | Iran49              |
| AAP3     | *L. tropica*         | CRI             | Iran49              |
|          | *L. donovani*        | LRM             | Spain166            |
| PGK      | *L. tropica*         | CRI             | Iran54              |
| MAPK     | *L. tropica*         | CRI             | Iran54              |
|          | *L. donovani*        | CRI             | India61             |
| PTP      | *L. tropica*         | CRI             | Iran54              |
| PTR1     | *L. major*           | LRM             | Canada154           |
|          | *L. braziliensis*    | LRM             | Brazil58            |
|          | *L. infantum*        | LRM             | Canada116           |
| TXNPx    | *L. infantum*        | CRI             | Algeria, Tunisia25   |
|          | *L. donovani*        | CRI             | India6               |
|          | *L. braziliensis*    | LRM             | Brazil155           |
|          | *L. major*           | LRM             | United Kingdom156    |
| KMP11    | *L. infantum*        | CRI             | France35             |
| Hsp70    | *L. braziliensis*    | CRI             | Brazil144           |
|          | *L. donovani*        | CRI             | India157            |
|          | *L. infantum*        | LRM             | Brazil158            |
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 India146 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.15 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 India150 and L. major21 and L. infantum in Spain19 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. infantum35 and in India on L. donovani.39 Histon H2A,153 Histon H139 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. major154 and L. infantum116 and L. braziliensis68 in Canada and Brazil, respectively.

Studies were conducted on the TXNPx gene in resistant strains isolated from patients in Algiers and Tunisia35 on L. infantum in India and L. donovani.76 Laboratory studies have been conducted on L. major and L. braziliensis in Brazil155 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. braziliensis144 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

### TABLE 1 (Continued)

| Gene | Leishmania species | Type of isolate | Country (reference) |
|------|-------------------|----------------|-------------------|
| GSH1 | L. guyanensis     | CRI            | Brazil159         |
|      | L. infantum       | CRI            | Tunisia35         |
|      | L. donovani       | CRI            | United Kingdom60  |
| TryR | L. major          | LRM            | Canada1           |
|      | L. donovani       | CRI            | India161         |
| Calcineurin | L. infantum  | CRI            | Iran92           |
| LRRs | L. infantum       | LRM            | Canada102        |
|      | L. donovani       | CRI            | India162         |
| LiMT and LiRos3 | L. infantum | CRI            | France105        |
|      | L. donovani       | LRM            | Spain103         |
|      | L. braziliensis   | CRI            | Peru163          |
|      | L. infantum       | LRM            | Germany106       |
|      | L. donovani       | CRI            | Germany106       |
|      | L. braziliensis   | CRI            | Peru163          |
|      | L. infantum       | LRM            | Canada23         |
|      | L. donovani       | CRI            | Germany128       |
|      | L. major          | LRM            | Canada128        |
|      | L. donovani       | CRI            | India132         |
|      | L. infantum       | LRM            | Brazil158        |
|      | L. braziliensis   | CRI            | Brazil158        |
| ARMS8, ARMS6, and HSP23 | L. infantum | LRM | Germany106 |
|      | L. donovani       | CRI            | Germany106       |
|      | L. braziliensis   | CRI            | Peru163          |
| Serine/threonine phosphatase protein | L. infantum | LRM | Canada23 |
| Iron superoxide dismutase A | L. infantum | LRM | Brazil125 |
|      | L. braziliensis   | LRM            | Brazil125        |
|      | L. donovani       | CRI            | India164         |
| Folate transporter 1 | L. infantum | LRM | Canada128 |
|      | L. major          | LRM            | Canada165        |
| HSP83 | L. donovani       | CRI            | India132         |
|      | L. infantum       | LRM            | Brazil158        |
|      | L. braziliensis   | CRI            | Brazil158        |
| SKCRP14.1 | L. donovani | CRI | India131 |
| LmACR2 | L. infantum | LRM | Canada134 |
|      | L. donovani       | LRM            | Canada134        |
| TDR1 | L. major          | LRM            | United Kingdom138 |

Abbreviations: CRI, clinical-resistant isolate; LRM, 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, Tunisia, and the United Kingdom, 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. Another study was conducted on *L. major* in Canada in in vitro condition.
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 India162 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 India.107 A survey of L. braziliensis 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. infantum128 and L. major165 from Canada.

HSP83 gene has been investigated as a biomarker of resistance in human specimens on L. donovani in India.33 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. donovani331 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), Histone 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), Histone 4 (L. major, L. infantum), TRPER (L. infantum), in India: AQ1P (L. donovani), ABCC3 (L. donovani), Pgps (L. donovani), Histone 4 (L. donovani), Histone H2A, Histone H1 (L. donovani), MAPK (L. donovani), TRPER (L. donovani), Hsp70 (L. donovani), Hsp70 (L. donovani), TryR (L. donovani), LRs (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 anthropo- notic 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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