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RESEARCH

Response and therapeutic failure to meglumine antimoniate, the first-line drug for cutaneous leishmaniasis infections in Colombian soldiers

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Abstract

Leishmaniasis is a vector-borne disease caused by *Leishmania* protozoa, transmitted through infected female phlebotomine sandflies. Cutaneous leishmaniasis (CL), its most common form, causes considerable morbidity, particularly among Colombian military personnel in endemic areas. Although meglumine antimoniate (MA) remains the first-line treatment, increasing reports of therapeutic failure (TF) raise concerns about its efficacy and highlight the need to identify associated risk factors. The objective of this study was to identify risk factors linked to MA treatment outcomes in Colombian soldiers with CL and to characterise the *Leishmania* species involved and their geographic distribution. A total of 128 soldiers diagnosed with CL (2018–2019) were followed for treatment response. Sociodemographic, clinical and lesion data were collected. *Leishmania* species were identified through HSP70 and MPI gene barcoding, and geographic origins were mapped. Selected isolates from TF patients underwent in vitro susceptibility testing to MA. The cure proportion was 67.9%, with TF in 32%. Factors significantly associated with TF included previous infections ($p=0.001$), prior MA use ($p=0.000$), lymphadenopathy ($p=0.008$) and lesion type ($p=0.002$). Multivariate analysis identified previous treatment ($p=0.000$), lesion size and infections acquired in the Orinoquía ($p=0.013$) and Pacific ($p=0.014$) regions as risk factors. *L. (V.) braziliensis* predominated, especially in Orinoquía and Amazon regions; *L. (V.) panamensis* was widespread, and *L. (L.) mexicana* appeared only in the Andean region. In vitro resistance to MA was not observed in analysed isolates; thus, this factor does not appear related to TF. TF is linked to specific clinical and epidemiological variables, supporting their integration into patient monitoring during MA therapy. Clinical trial number: not applicable

Keywords Cutaneous leishmaniasis · Meglumine antimoniate · Glucantime · *Leishmania (Viannia) braziliensis* · *Leishmania (Viannia) panamensis* · *Leishmania (Leishmania) amazonensis* · *L. (Leishmania) mexicana* · Therapeutic failure

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Introduction

Leishmaniasis is a vector-borne disease caused by protozoa of the *Leishmania* genus, transmitted through the bites of infected female phlebotomine sandflies. More than twenty species infect humans, producing three main clinical forms: cutaneous, mucocutaneous, and visceral leishmaniasis (Panamerical Health Organization and and Organization 2019; Steverding 2017). Cutaneous leishmaniasis (CL) is the most prevalent, with between 700,000 and one million new cases annually in over ninety endemic countries, predominantly affecting vulnerable populations in low-resource and conflict-affected areas. Although CL is rarely life-threatening, it causes chronic ulcers, permanent scarring, and notable psychological and social consequences. Its contribution to the global disease burden, measured in disability-adjusted life years (DALYs), reflects long-term impacts on health and social integration. Visible lesions, particularly on the face or limbs, often lead to stigma, social exclusion, and reduced quality of life (Okwor and Uzonna 2016).

In Colombia, CL accounts for approximately 98% of all reported cases of leishmaniasis. *Leishmania (Viannia) braziliensis* and *L. (V.) panamensis* are the predominant causative agents, with *L. (Leishmania) mexicana* reported in some regions (Patiño et al. 2017). The Colombian National Institute of Health reported around 6,000 new CL cases annually between 2018 and 2023, confirming sustained endemic transmission (Sivigila Instituto Nacional de Salud 2025). Colombian military personnel are at particularly high risk due to deployments in rural and jungle areas, with 1,488 and 1,588 cases reported in 2018 and 2019 by the Army Health Directorate (DISAN). The documented presence of competent sandfly vectors (*Lutzomyia*, *Psychodopygus*, *Nyssomyia spp*, and *Pintomyia* spp.) and animal reservoirs in these areas supports the persistence of active transmission (Ovalle-Bracho et al. 2019; Posada-López et al. 2023).

Treatment of CL in military personnel follows Colombia's 2010 national guidelines, which recommend meglumine antimoniate (Glucantime®) as first-line therapy (Ministerio de la Protección Social. República de Colombia. Instituto Nacional de Salud. Organización Panamericana de la Salud. 2010). The standard regimen consists of intramuscular administration of 20 mg/kg/day for 20 consecutive days. Treatment response is typically assessed through clinical evaluation, including lesion resolution and progression monitoring, and, when possible, supported by laboratory confirmation. Although MA remains the cornerstone of CL management in Colombia, recent reports indicate an increasing number of cases with poor or incomplete therapeutic response.

Rising rates of TF following MA treatment have been reported in several endemic regions worldwide, including Asia and South America (Llanos-Cuentas et al. 2008; Munir et al. 2008; Haldar et al. 2011; Rubiano et al. 2012; Walker et al. 2012; Perez-Franco et al. 2016; Calvopiña et al. 2017; Castro et al. 2017; Jaffar et al. 2018; Bamorovat et al. 2018; Aflatoonian et al. 2019; Amorim et al. 2019; Carvalho et al. 2019; García-Bustos et al. 2021; Nahidi et al. 2023; Fernández et al. 2024;). Approximately 10% of cases treated with MA in the Colombian military population result in therapeutic failure. In 2018 and 2019, DISAN reported 141 and 158 such cases, respectively. These patterns highlight the need for a better understanding of the determinants of treatment failure in this population.

TF is a multifactorial process influenced by host, drug, and parasite-related factors. Patient determinants include age, immune status, infection duration, lesion number and location, pharmacogenetic variability, and adherence to treatment (Ponte-Sucre et al. 2017; Conceição-Silva et al. 2018; Wijnant et al. 2022; Gómez et al. 2024). Drug formulation, pharmacokinetics, and dosing schedule may also affect treatment efficacy. Parasite characteristics, such as species-specific susceptibility and genetic adaptations, further influence outcomes. Although drug resistance (DR) is an important contributor, it is not synonymous with TF, and persistent parasites after treatment failure may facilitate DR emergence (Fernández et al. 2014; Locatelli et al. 2014).

Approximately 25%–40% of TF cases in Colombia, Argentina, Brazil, Peru, and Guatemala have been associated with *L. (V.) braziliensis* (Romero et al. 2001; Arevalo et al. 2007; Vélez et al. 2015; Adaui et al. 2016; Perez-Franco et al. 2016; Berbert et al. 2018; Carvalho et al. 2019; Rugani et al. 2019;), whereas 15%–31% have been associated with *L. (V.) panamensis* (Teixeira et al. 2008; Castro et al. 2017; Fernández et al. 2024; Rubiano et al. 2012).

Given the persistent burden of CL among Colombian soldiers and the emerging threat of therapeutic failure, longitudinal studies assessing disease dynamics, *Leishmania* species distribution, and treatment outcomes are urgently needed. Such information is vital to guide clinical management and strengthen public health interventions in endemic areas. This study aimed to characterise the sociodemographic and clinical profiles of affected soldiers, identify circulating *Leishmania* species and their distribution, and analyse therapeutic response and factors associated with failure to improve CL management in Colombia.

Materials and methods

Study population and ethical considerations

This descriptive cross-sectional study included 128 soldiers from the Colombian National Army who had a clinical and

parasitological diagnosis of CL, confirmed by direct smear, culture of lesion aspirate, tissue biopsy, and polymerase chain reaction (PCR). Participants were recruited between 2018 and 2019 from two military healthcare centers: The Leishmaniasis Recovery Centre in Bonza, Boyacá ($n=101$) and the BASAN Health Battalion ($n=27$). Clinical records and associated patient data are securely stored at the healthcare institutions where the participants received medical care. Owing to the sensitive nature of the information and to protect participant privacy, these data are not publicly available. Access to the datasets may be granted upon reasonable request and with appropriate ethical and institutional approval.

All participants provided informed consent by signing a form approved by the Ethics Committee of the Central Military Hospital (Approval No. 35420; dated November 26, 2015). A standardized registration form was used to collect general, clinical, and epidemiological data, including: sex; place and date of birth; use of insect repellents and protective awnings; history of previous *Leishmania* infections (defined as fully healed lesions confirmed by medical evaluation, with at least a 2-year documented clinical history); prior treatments; deployment location at the time of diagnosis; signs and symptoms suggestive of mucocutaneous leishmaniasis; any other diseases or infections within the past year; and diagnostic tests performed for the current infection.

The study was conducted in compliance with international and national ethical guidelines, including the Declaration of Helsinki (World Medical Association), the Belmont Report, the CIOMS Guidelines, and Colombian regulations specifically Resolution 8430 of 1993, which outlines the scientific, technical, and administrative standards for health research. Soldiers are considered a vulnerable population due to their hierarchical and subordinate status; therefore, additional ethical safeguards were required. Therefore, special authorisation was obtained from the Army Command to conduct the study.

Samples

Cutaneous leishmaniasis was diagnosed in each participant using parasitological methods, confirmed according to the case by direct smear, culture of lesion aspirate, tissue biopsy, and polymerase chain reaction (PCR). Key clinical features, such as location, number, size, and type of lesions, were documented. Samples were obtained by aspirating lymph and tissue from the lesions. Before sampling, the lesions were cleaned with saline to remove scabs and disinfected with an alcohol/iodine solution. A single sample was collected from multiple points along the lesion margin using a 30G × 13 mm needle and a 1 ml syringe filled with 60 units of sterile solution (1% glucose and 0.85% sodium chloride). The samples were transported to the laboratory within a maximum of 4 h. The aspirated material was homogenised in the solution and divided for parasite culture and DNA extraction for identification of *Leishmania* species. The aliquot for DNA extraction was placed in a screw-cap vial and stored at 70 °C until processing, which occurred within 48 h after sample collection. For culture, the sample was inoculated into culture medium within approximately 4 h of collection.

Follow-up of response to Meglumine antimoniate treatment

Participants were monitored to evaluate their response to MA and to assess clinical progression at three time points: day 0 (baseline), day 15 of treatment (follow-up 1), and 15 days after completing treatment (follow-up 2) (see Fig. 1; Table 1). Patients with cardiovascular, renal, or hepatic diseases were excluded, as the absence of these conditions was required to initiate treatment. In line with "the Guide for the Comprehensive Clinical Care of Patients with Leishmaniasis", (Ministerio de la Protección Social. República de Colombia. Instituto Nacional de Salud. Organización Panamericana de la Salud, 2010), all patients received MA at a dose of 20 mg/kg/day, administered as a single daily intramuscular injection for 20 consecutive days.

During follow-up assessments, clinical cure was defined as complete healing of the lesion, including full epithelialization, flattening of the active edge of the ulcer, disappearance of induration at the base, scar formation, and resolution of any lymphadenopathy. The treating physician evaluated lymphadenopathy clinically through physical examination, specifically by palpation of affected lymph nodes to monitor reduction in size, pain, and consistency until resolution. These evaluations were carried out at specialised healthcare centres for leishmaniasis management, namely the Leishmaniasis Rehabilitation Centre of the Health Battalion in Bogotá and the National Leishmaniasis Rehabilitation Centre in Duitama, Boyacá. TF was diagnosed by the treating physician when there was no clinical or parasitological improvement. This was characterised by minimal or no reduction in lesion size, persistence or enlargement of the lesion, a positive result in at least one parasitological test (direct smear, culture, PCR, biopsy), and lymphadenopathy continued for 30 days after treatment completion.

On day 0 (baseline follow-up), a clinical and epidemiological survey was conducted, and lesions were identified and described. Information collected included the lesion's body location, duration, size, type (ulcer, papule, plaque, or nodule), and distribution (localised or disseminated). The assessment also included checking for possible co-infections

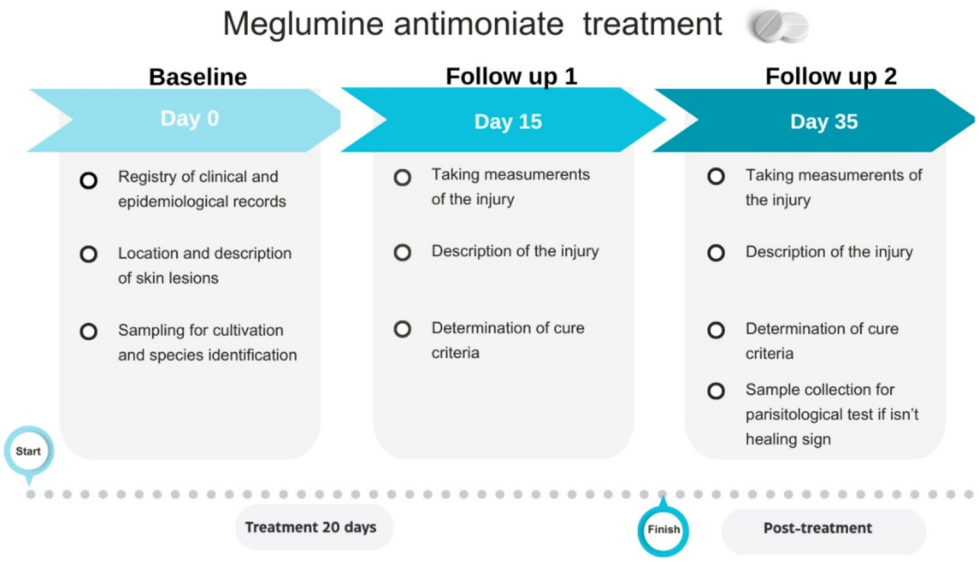

Fig. 1 Follow-up during treatment. Three time-points were realised to determine the parasitic cure with the use of MA

Table 1 Evolution of lesions in patients with parasitological cure during follow-up

Clinical criteria for treatment	Basal control		Control 1		Control 2		Total	
	n = 87	(%)	*n* = 87	(%)	*n* = 87	(%)	*n* = 87	(%)
Total lesion epithelialization	0	0.0	16	18.4	4	4.6	20	23.0
Flattening of the ulcer's active edge	1	1.1	3	3.4	2	2.3 6		6.9
Disappearance of the base induration	1	1.1	5	5.7	0	0.0	6	6.9
Lesion healing	0	0	4	4.6	48	55.2	52	59.8

(such as fungi or bacteria), regional lymphadenopathy, and signs or symptoms indicative of MCL. Additionally, an aspirate sample was collected from the lesion for parasite culture and species identification using end-point polymerase chain reaction (PCR).

On day 15 of treatment (follow-up 1), after completing the full course of anti-leishmanial therapy, lesion size and appearance were reassessed. Healing was evaluated based on established criteria.

On day 35 post-treatment (follow-up 2), the clinical assessment was repeated. If healing was incomplete, a new parasitological test was performed to confirm the presence of *Leishmania* parasites.

Culture of the *Leishmania* parasite

Leishmania parasites were isolated following the protocol described in (Beltrán et al. 1988). An aliquot of the lesion aspirate was inoculated into a tube containing Novy-Mac-Neal-Nicolle medium and incubated at 25 °C. After 5–6 days of incubation, approximately 5 µL of culture was extracted and examined under a microscope to detect the presence of flagellated promastigote. Positive cultures were then expanded by transferring the parasites to Schneider's Insect Medium supplemented with 20% heat-inactivated fetal bovine serum (FBS), penicillin (100 U/mL), and streptomycin (100 µg/mL). The *Leishmania* parasites were incubated at 26 °C and monitored every 2–3 days under an inverted microscope until a concentration of 5×10^6 parasites/µL. An aliquot of 5 µL was taken to verify the expected concentration.

Identification of *Leishmania* species

DNA was extracted from the aspirate subsample using the commercial Invisorb Spin Universal kit (STRATEC®, Berlin, Germany) according to the manufacturer's instructions. DNA concentrations were measured using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific Inc, Waltham, MA, USA). Samples with concentrations of 20 ng/µL or higher were accepted. Purity was evaluated using absorbance ratios: 260/230 values between 1.0 and 2.0 indicated minimal contamination from salts, phenols, or carbohydrates, while 260/280 values below 2.0 indicated low levels of proteins, RNA, or aromatic compounds. DNA quality and integrity were further verified by running the samples on 1% agarose gels.

For species identification, the extracted DNA was PCR targeting two genes: mannose phosphate isomerase (*mpi*) using primers MPI-32 F (5'-CADGCGGTACAGSSAA-AAGT-3') and MPI-32R (5'-ACCACAAGCCWGAGCT-CATT-3'), and heat shock protein 70 (*hsp70*) using primers HSP70F (5'-AGGTGAAGGCGACGAACG-3') and HSP70R (5'-CGCTTGTCCATCTTTGCGTC-3') (33). The reaction conditions and thermal profiles were followed

as previously described (Hernández et al. 2014). Amplification was confirmed by electrophoresis on 1% agarose gels stained with SYBR Safe. PCR products were then purified and sequenced by Macrogen (Seoul, South Korea) using the Sanger method with BigDye Terminator v3.1 chemistry on an ABI 3730xl DNA analyser. The sequences obtained were compared against GenBank *Leishmania* entries using BLASTn on the NCBI platform, with a minimum identity cutoff of 98% (Patino et al. 2017; Rigg et al. 2019; Miranda et al. 2021). The nucleotide sequences were deposited in the European Nucleotide Archive of the European Bioinformatics Institute under the project accession numbers PRJEB77615 (ERZ24812769) for the HSP70 gene and PRJEB77616 (ERZ24812770) for the MPI gene.

Phylogenetic inferences

The sequences were aligned using MAFFT v7.48 (Katoh and Frith 2012) with reference sequences of the HSP70 gene sequences of multiple *Leishmania* species:

Leishmania amazonensis strain HSP70 (MG029123.1).
Leishmania mexicana partial HSP70 (HF586413.1).
Leishmania tropica voucher ISS3183 HSP70 (MK335938.1).
Leishmania aethiopica partial HSP70 (HF586411.1).
Leishmania major partial HSP70 (HF586403.1).
Leishmania donovani strain HSP70 (JX312712.1).
Leishmania infantum heat shock protein HSP70 (KX759517.1).
Leishmania chagasi partial HSP70 (FN395037.1).
Leishmania lainsoni strain HSP70 (GU071174.1).
Leishmania lindenbergi strain HSP70 (PP331244.1).
Leishmania guyanensis isolate HSP70 (KX574011.1).
Leishmania panamensis isolate HSP70 (KX574010.1).
Leishmania braziliensis HSP70 (ON806909.1).
Leishmania peruviana isolate HSP70 (KX573935.1).

The *hsp70* mRNA sequence of *Trypanosoma cruzi* (accession number X67716.1) was used as the outgroup. A maximum likelihood (ML) phylogenetic tree was generated using IQ-TREE 2 (version 1.6) (Minh et al. 2020), with node support evaluated by 1,000 bootstrap replicates. The final tree was visualized and illustrated using Interactive Tree of Life (iTOL) version 4 (http://itol.embl.de) (Letunic and Bork 2019).

Phenotypes resistant to meglumine antimoniate

Cultures containing 1×10^6 logarithmic-phase promastigotes were incubated at 26 °C for 72 h in a 5% CO_2 environment with varying concentrations of SbIII (200–1.5 mg/mL). Susceptibility to SbIII was assessed by calculating the EC_{50} a colourimetric MTT assay [3-(4,5-dimethylthiazol-2-yl)−2,5-diphenyltetrazolium bromide], as previously described (Paris et al. 2007). Briefly, 10–20 µL of a stock MTT solution (5 mg/mL) was added to each well and incubated at 26 °C for 3 h. Subsequently, 100 µL of the stop solution (acid-isopropanol) was added to dissolve the formed dark blue formazan crystals. The absorbance was measured at 570 nm using a Tecan GENios Microplate Reader (Biotek, Winooski, VT, USA). EC_{50} values were calculated using GraphPad Prism v5.0 software. All assays were performed in triplicate. Parasites without SbIII exposure served as negative controls, whereas those treated with amphotericin B (5000 µg/mL) served as positive controls. Promastigotes of *L. braziliensis* (MHOM/BR75/M2904) and *L. panamensis* (MHOM/COL/81/L13) sensitive to SbIII (SSG_S) and resistant to SbIII (SSG_R) were used as controls (https://www.nature.com/articles/s41598-019-45538-9).

Statistical analyses

The categorical variables were described using counts and percentages. Quantitative variables were tested for normality using the Shapiro-Wilk test. Normally distributed variables are reported as means with standard deviations, whereas non-normal variables are presented as means with interquartile ranges (IQR). Group differences in proportions were assessed using Pearson's chi-square test or Fisher's exact test. Due to non-normal distribution, numerical variables were compared using the Mann–Whitney U test. Bivariate analyses were performed to calculate the crude odds ratios (ORs). Variables with clinical or statistical significance were included in the multivariate logistic regression model. Statistical significance was set at $p < 0.05$. The Hosmer Lemeshow test was used to evaluate the model fit. All analyses were conducted using Stata version 17.

Results

Response to the meglumine antimoniate

Among the 128 patients diagnosed with cutaneous leishmaniasis, 67.9% (87 patients) had a successful response to MA, whereas 32.0% (41 patients) had treatment failure. The clinical improvement of skin lesions observed during follow-up visits (Table 1), along with the resolution of lymphadenopathy, when present, was defined as parasitological cure.

Clinical and epidemiological characteristics of the therapeutic outcome groups

Table 2 presents the clinical and epidemiological characteristics of soldiers with PC and TF. The main findings are summarised below. The mean age was 23 years in the PC and TF groups. Soldiers came from various regions of the country, with no statistically significant difference in geographic origin between the two groups ($p=0.020$; χ^2 test). Most infections occurred in the Colombian Amazon region,

Table 2 Sociodemographic and clinical characteristics of patients with CL

Characteristics	TF cases		PC cases		*p* value (<0.05)
	n=41	%	*n*=87	%	
Sociodemographic					
Age (mean, IQR)	23 (21–27)	-	23 (21–25)	-	0.3358 §
Region where the infection was acquired					0.020 χ*
Caribbean	5	12.2	1	1.1	
Andean	6	14.6	11	12.6	
Orinoquia	4	9.8	8	9.2	
Pacific	6	14.6	30	34.5	
Amazon	19	46.3	33	37.9	
ND	1	2.4	4	4.6	
Length of stay in the region where the infection was acquired (IQR)	5 (6-3.75) - Months		4.5 (6−3) months	-	0.2179 §
Prevention measures					
Netting	33	80.5	65	74.7	0.070 χ
Repellent	25	61.0	55	63.2	0.774 χ
History of leishmaniasis					
Previous infections					0.001 § **
0	11	26.8	66	75.9	
1	11	26.8	7	8.0	
2	4	9.8	0	0	
3	1	2.4	0	1.1	
4	1	2.4	1	1.1	
ND	13	31.7	12	13.8	
Previous treatment with meglumine antimoniate	19	46.3	11	12.6	0.000χ***
Associated clinic					
Lymphadenopathy	9	22.0	9	10.3	0.008 § **
Number of lesions					0.709 χ
1	36	81.8	68	76.4	
2	4	9.0	16	36.3	
3	2	4.5	3	6.8	
4	1	2.2	1	2.2	
5	1	2.2	1	2.2	
Location of the lesions					0.664 χ
Upper limb	23	56.1	51	58.6	
Lower limb	7	17.1	10	11.5	
Trunk or back	3	7.3	3	3.4	
Face, ear, or neck	7	17.1	17	19.5	
ND	1	2.4	6	6.9	
Lesion type					0.002 χ **
Ulcer	31	75.6	86	98.9	
Papule	3	7.3	0	0	
Plaque	2	4.9	0	0	
Warty	1	2.4	0	0	
ND	4	9.8	1	1.1	
Lesion size (mean IQR)	2.7(9−1) cm ^{2}		1.74 (4−0,8) cm ^{2}		0.0180 §

Age: expressed in years, ND: not data

§ Wilcoxon rank-sum (Mann-Whitney)

χ Chi-square test

** -*** highly significant

affecting 37.9% (33/87) of the PC group and 46.3% (19/41) of the TF group. The Pacific region was the second most frequent area of exposure, reported in 34.5% (30/87) of PC cases and 14.6% (6/41) of TF cases. The duration of deployment in endemic areas was similar between the groups, with a mean stay of 5 and 4.5 months in the TF group and 4.5 months in the PC group ($p=0.2179$; Wilcoxon rank-sum test [Mann–Whitney]).

A history of previous *Leishmania* infection was significantly more frequent among patients with TF ($p=0.000$; Wilcoxon rank-sum test [Mann–Whitney]). In the TF group, 26.8% (11/41) reported one prior episode, 9.8% (4/41) had two previous episodes, and one patient each (2.4%) had experienced three or four episodes. In contrast, only 8.0% (7/87) of patients in the PC group reported a single prior infection, whereas one patient each reported three or four episodes (1.1%).

A significantly higher proportion of TF patients 46.3% (19/41) had previously been treated with MA compared with 12.6% (11/87) of patients who achieved PC ($p=0.000$; χ^2 test).

Lymphadenopathy was observed in 22.0% (9/41) of patients with TF, compared with 10.3% (9/87) of those with PC ($p=0.008$; χ^2 test). The number and location of lesions varied among individuals, with the upper limbs being the most commonly affected site in both groups ($p=0.664$; χ^2 test).

Ulcers were the only type of lesion present in patients with PC and were also found in 75.6% (31/41) of patients with TF Other lesion types, including papules, plaques, and warty lesions, were observed exclusively in the TF group, a difference that was statistically significant ($p=0.002$; χ^2 test).

In terms of lesion size, TF patients had a larger mean lesion area (2.7 cm²) compared to PC patients (mean = 1.74 cm²). However, this difference was not statistically significant ($p=0.0180$; Wilcoxon rank-sum test [Mann–Whitney]).

During their deployment, none of the soldiers reported a diagnosis of other vector-borne diseases, such as Chagas disease. In addition, no participants exhibited clinical signs of mucosal involvement or mucocutaneous leishmaniasis.

Logistic regression

Variables strongly linked to TF, including prior treatment with MA and the geographic region of infection were analysed using both bivariate (crude odds ratio [cOR]) and multivariate (adjusted odds ratio [aOR]) logistic regression models, with statistical significance set at $p \leq 0.05$ (Table 3). Due to the limited sample size, lymphadenopathy and previous infection history were excluded from the final model. The lesion area was included to evaluate its possible effect on treatment outcomes. Of the 128 patients, 8 were excluded from the final model due to missing data. The multivariate analysis identified the following risk factors for TF: previous MA treatment ($p = 0.000$), lesion area ($p = 0.086$), and infection acquired in the Orinoquía ($p = 0.013$) or Pacific ($p = 0.014$) regions.

Identification of *Leishmania* species and phylogenetic inference

Leishmania species were identified in 83.5% (101 out of 128) of the soldiers, with 77.2% (78/101) belonging to the PC group and 22.7% (23/101) to the TFTF group. In 21.0% (27/128) of cases, species identification was unsuccessful, likely due to low parasite load in aspirate samples that impaired PCR amplification.

Species identification and phylogenetic analysis were conducted by sequencing partial fragments of the *mpi* and *hsp70* genes. The *mpi* gene successfully identified species in 74 samples (71.2%), whereas sequencing of the *hsp70* gene produced results for all 101 samples (100%). BLASTn analysis against the GenBank database confirmed an average sequence identity of 98% with known *Leishmania* species.

Leishmania (Viannia) braziliensis was identified in 58 samples (56.8*%)*, *L. (V.) panamensis* in 39 samples (38.2%), and *L. (Leishmania) mexicana* in 4 samples (3.9%). These identifications were based on the consensus results of *mpi* and *hsp70* typing. The *hsp70* gene sequences were aligned and used for robust phylogenetic reconstruction. As shown in Fig. 2, the tree topology revealed five well-supported clusters (bootstrap ≥ 90.0), each corresponding to distinct *Leishmania* species or species complexes: Cluster 1 included sequences of *L. mexicana* and *L. amazonensis*,

Table 3 BV and MV analysis of factors associated with leishmaniasis in the TF group

Factor	Bivariate analysis				Multivariate analysis			
The region where the infection was acquired	cOR	95% CI		*p*	aOR	95% CI		*p value (< 0,05)*
Andean	0,11	0,01	1,16	0,066	0,18	0,01	2,39	0,193
Orinoquía	0,10	0,01	1,17	0,067	0,03	0,00	0,47	0,013
Pacific	0,04	0,00	0,41	0,007	0,04	0,00	0,52	0,014
Amazon	0,12	0,13	1,06	0,056	0,14	0,01	1,51	0,104
Lesion size (cm^2)	1,09	1,02	1,17	0,013	1,09	0,99	1,16	0,086
Previous treatment with mealtime antomoniate	5,97	2,47	1,44	0,000	11.03	3,46	35,10	0,000

cOR: Crude odds ratio. aOR: Adjusted Odds Ratios. CI: confidence intervals

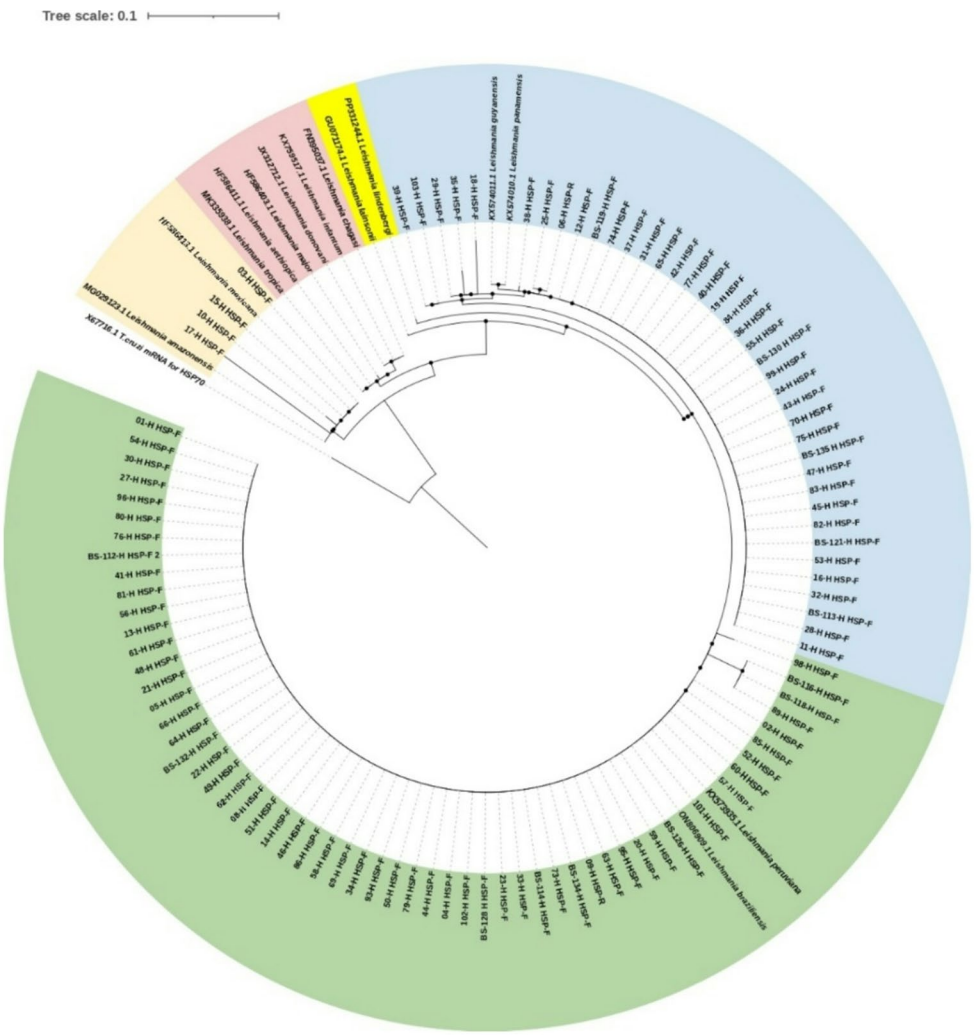

Fig. 2 Phylogenetic reconstruction of the hsp70 sequences. Phylogenetic reconstruction of 101 *hsp70* gene sequences obtained from CL study population samples and the reference strain retrieved from GenBank. *L. mexicana/L. amazonensis* (light orange), Old World *Leishmania* species (light pink), *L. lainsoni* and *L. lindenbergi* (yellow), *L. panamensis/L. guyanensis* (light blue), *and L. braziliensis/L.* peruviana (green). The numbers represent each sample's coding

Cluster 2 grouped sequences of Old World *Leishmania* species, Cluster 3 comprised sequences of *L. lainsoni* and *L. lindenbergi*, Cluster 4 included sequences of *L. panamensis* and *L. guyanensis* and Cluster 5 included sequences of *L. braziliensis/L. peruviana*.The analysis also revealed the presence of distinct genotypes within both the *L. (V.) braziliensis* and *L. (V.) panamensis* clusters.

L. (V.) braziliensis was the predominant species in both patient groups. It was identified in 56.4% (44/78) and 60.8% (14/23) of patients with PC and TF, respectively. The second most common species was *L. (V.) panamensis*, found in 39.7% (31/78) of PC patients and 34.7% (8/23) of TF patients. *L. (L.) mexicana* was less frequent, detected in 3.9% (3/78) of PC patients and 4.3% (1/23) of TF patients. No co-infections with more than one *Leishmania spp*. were identified among the analysed patients (Table 4).

Analysis of the association between *Leishmania* species and clinical features in patients with TF showed no statistically significant differences between lesion type and infecting species ($p = 0.725$; χ^2 test). However, plaque-like lesions were found exclusively in patients infected with *L. (V.) panamensis*, whereas warty lesions were found only in those infected with *L. (V.) braziliensis* (Table 4).

Geographical distribution of *Leishmania* species

As shown in Fig. 3, *L. (V.) braziliensis* was mainly identified in the Colombian Amazon and Orinoco regions, with the departments of Guaviare and Meta reporting the highest number of cases. It was also found, though less commonly, in the Andean (Boyacá and Norte de Santander), the Pacific region (Nariño), and Caribbean (La Guajira and Atlántico) regions. In contrast, *L. (V.) panamensis* was primarily distributed across the Andean region, followed by the Caribbean and Pacific regions and, to a lesser degree, in the Amazon. Within the Amazon, most cases were concentrated in the Putumayo department. In the case of *L. (L.) mexicana*, which had a lower number of cases, three were identified in Norte de Santander and one in the department of Bolívar.

Table 4 *Leishmania* species identified in patients with CL

Leishmania infection species	*L. (L.) Mexicana* *n*=4	%	*L. (V.) braziliensis* *n*=58	%	*L. (V.) panamensis* *n*=39	%	ND *n*=24	%
Cases of response (PC)*	3		44		31		7	
Previous infections 0	3	100	33	76.7	27	87.1	3	42.8
1	0	0.0	5	11.3	1	3.2	1	14.2
2	0	0.0	0	0.0	0	0.0	0	0.0
3	0	0.0	0	0.0	0	0.0	0	0.0
4	0	0.0	1	2.2	0	0.0	3	0.0
ND	0	0.0	5	11.3	3	9.6	7	42.8
Lesion form								
Ulcer	3	100.0	42	97.6	31	100.0	7	100.0
Papule	0	0	0	0	0	0	0	0
Plaque	0	0	0	0	0	0	0	0
Warty	0	0	0	0	0	0	0	0
ND	0	0	1	2.3	0	0	7	100.0
Therapeutic failure (TF)*	1		14		8		7 17	
Previous infections 0	1	100	4	28.5	3	37.5	2	11.7
1	0	0.0	1	7.1	2	25	8	47.0
2	0	0.0	3	21.4	0	0.0	1	5.8
3	0	0.0	1	7.1	0	0.0	0	0.0
4	0	0.0	0	0.0	0	0.0	1	5.8
ND	0	0.0	5	35.7	3	37.5	5	29.4
Lesion form								
Ulcer	1	100.0	11	78.5	6	75.0	12	100.0
Papule	0	0	0	0	2	25.0	1	0
Plaque	0	0	1	7.1	0	0	1	0
Warty	0	0	1	7.1	0	0	0	0
ND	1	100.0	1	7.1	0	0	3	17.6

Susceptibility test of isolated *Leishmania* strains to meglumine antimoniate

Leishmania parasites were successfully cultured from 34.1% (14 of 41) of patients with TF. Of these isolates, 38.4% (5/13) were *L. (V.) braziliensis*, 61.5% (8/13) were *L. (V.) panamensis*, and one isolate (7.6%) was *L. (L.) mexicana.*

For *L. (V.) panamensis*, EC_{50} values ranged from 0.5 to 4.5 µg/mL, which is within the range of the drug-sensitive reference strain (3.5 µg/mL). In the case of *L. (V.) braziliensis*, EC_{50} values ranged from 0.6 to 3.9 µg/mL, showing some variability but remaining close to the drug-sensitive reference strain (2.8 µg/mL). For *L. (L.) mexicana*, the single isolate showed an EC_{50} of 5.2 µg/mL, which is higher than the reference values for *L. (V.) braziliensis* and *L. (V.) panamensis* (Table 5). All tested isolates exhibited an in vitro drug-sensitive profile, suggesting that TF in these patients is likely attributable to factors other than parasite resistance.

Discussion

Response rate to meglumine antimoniate and related clinical factors

In this study, 67.9% (87/128) of Colombian soldiers diagnosed with cutaneous leishmaniasis responded successfully to MA treatment, a proportion that is consistent with previously reported efficacy levels of approximately 75%. However, TF occurred in 32.0% (41/128) of the patients, representing a notable increase compared with earlier reports in the Colombian population. These findings are in line with a previous study conducted in a similar military cohort between 2017 and 2019, which reported a TF rate of 35.7% (35/98) following first-line MA treatment (Correa-Cárdenas et al. 2020). In our study, TF exceeded the proportions reported in most Latin American countries, which generally range from 7% to 24%, except in Argentina (47.9%) and Brazil (up to 60%) (Llanos-Cuentas et al. 2008; Brito et al. 2017; Calvopiña et al. 2017; Berbert et al. 2018;

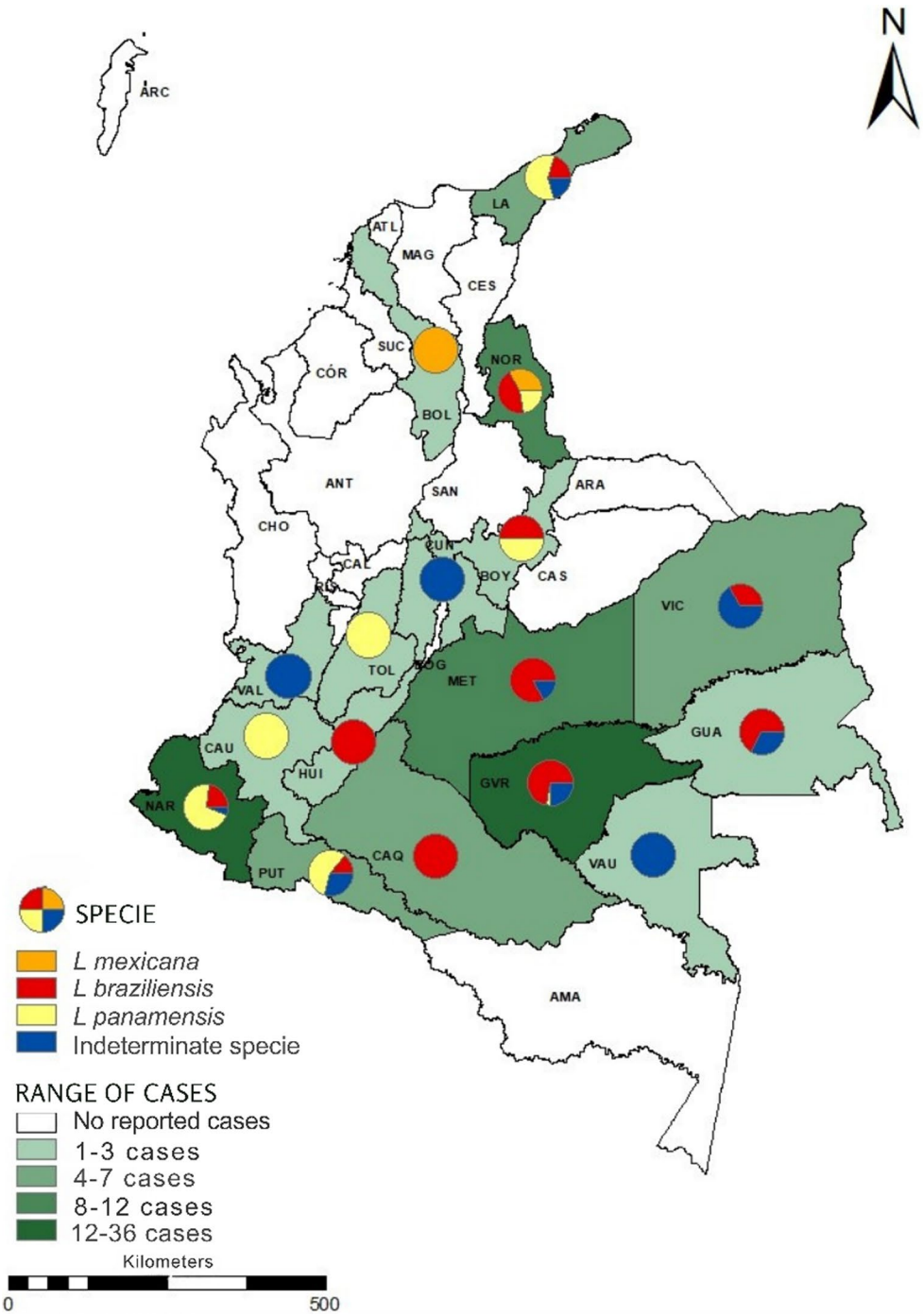

Fig. 3 Geographical distribution of *Leishmania* isolates and cases of CL in the study population. The shades of green represent the range of cases, and the circles represent the *Leishmania* species in different regions

Amorim et al. 2019; Carvalho et al. 2019; García-Bustos et al. 2021). In Colombia, a recent study reported a 43% TF among patients infected with *L. (V) panamensis*, mainly associated with risk factors such as prolonged lesion duration, age-related metabolic variation, poor treatment adherence, and lesions on cartilaginous areas impairing drug penetration (Fernández et al. 2024).

Our sociodemographic analysis revealed no significant differences between the parasitological cure and TF groups. However, multivariate analysis showed a strong association between TF and prior MA treatment. Additional risk factors included larger lesion size and infection acquired in the Orinoquía and Pacific regions of Colombia, consistent with previous studies highlighting prior antimonial treatment as a persistent and significant predictor of TF (Rodrigues et al. 2006; Munir et al. 2008; Mohammadzadeh et al. 2013; Martínez-Valencia et al. 2017; Amorim et al. 2019; García-Bustos et al. 2021).

Table 5 EC_{50} to Meglumine antimoniate in *Leishmania* isolates of soldiers with CL

Strain	Group of patients	Species	EC_{50}(µg/mL)
1	PC	*L. (V.) braziliensis*	3.9
2	TF	*L. (V.) braziliensis*	0.9
3	TF	*L. (V.) braziliensis*	0.6
4	TF	*L. (V.) braziliensis*	0.7
5	TF	*L. (V.) braziliensis*	3.1
6	TF	*L. (V.) braziliensis*	2.6
7	TF	*L. (V.) panamensis*	1.7
8	TF	*L. (V.) panamensis*	0.5
9	TF	*L. (V.) panamensis*	4.5
10	TF	*L. (V.) panamensis*	2.2
11	TF	*L. (V.) panamensis*	0.7
12	TF	*L. (V.) panamensis*	2.1
13	TF	*L. (V.) panamensis*	3.6
14	TF	*L. (V.) panamensis*	1.5
15	TF	*L. (L) mexicana*	5.2
Control S *Lp*	Wildtype	*L. (V.) panamensis*	3.5
Control S *Lb*	strains**	*L. (V.) braziliensis*	2.8
Control R *Lp*	Resistant	*L. (V.) panamensis*	51.9
Control R *Lb*	Strains*	*L. (V.) braziliensis*	42.0

*Strains resistant to meglumine antimoniate

**Strains sensitive to meglumine antimoniate

PC = Parasitological cure

TF = Therapeutic failure

Leishmania parasites may persist in host tissues for years after clinical healing, allowing reactivation when host–parasite balance is disrupted. Such persistence can enhance parasite virulence, weaken host immunity, and increase the risk of treatment failure, especially in individuals receiving repeated courses of the same drug (Morgado et al. 2010; Castro et al. 2017; Martínez-Valencia et al. 2017; Conceição-Silva et al. 2018).

García et al. (2021) also demonstrated that the geographical origin of infection significantly influences therapeutic outcome. In their study of 71 patients with cutaneous and mucosal leishmaniasis treated with MA, TF remained strongly associated with the region of exposure after multivariate adjustment ($p=0.036$; adjusted OR=8.06), supporting our findings(Carvalho et al. 2019; García-Bustos et al. 2021).

Lymphadenopathy was more frequently observed in the TF group, although it was excluded from the multivariate analysis due to the limited sample size. Nevertheless, lymphadenopathy has been proposed as a potential indicator of increased disease severity and parasite dissemination through the lymphatic (Mendonça et al. 2004; Castro et al. 2017; Nahidi et al. 2023).

Identification and geographical distribution of *Leishmania* species

In our study, the most frequently identified species were *L. (V.) braziliensis* and *L. (V.) panamensis*, consistent with previous reports identifying them as the main causes of cutaneous leishmaniasis in Colombia (Ramírez et al. 2016; Ovalle-Bracho et al. 2019; Correa-Cárdenas et al. 2020; Fernández et al. 2024). *L. (V.) braziliensis* predominated among patients with TF, corroborating earlier studies linking this species to poorer treatment outcomes. Nevertheless, its presence in several cured cases likely reflects its broad distribution in endemic areas TF was also associated with *L. (V.) panamensis*, a species less frequently connected to drug resistance in Colombia (Castro et al. 2017; Fernández et al. 2024). However, Fernández et al. (2024) reported that *L. (V.) panamensis* strains of zymodeme 2.3 were linked to larger lesions and higher parasite loads in mice, suggesting greater pathogenicity and reduced treatment responsiveness. These findings highlight the need for further research on treatment outcomes across *Leishmania* genotypes and the clinical relevance of naturally resistant strains (Fernández et al. 2024).

We identified four cases of *L. mexicana* in the military population, with no infections by other species such as *L. guyanensis*, *L. amazonensis*, *L. lainsoni*, *L. naiffi*, *L. lindenbergi*, or *L. infantum*, consistent with previous reports in Colombia (Patino et al. 2017; Correa-Cárdenas et al. 2020). No co-infections were detected, possibly due to the limited sample size or study exclusion criteria. The species distribution observed aligns with earlier studies in military populations.

L. (V.) braziliensis was predominantly found in the Orinoco and Amazon regions, while *L. (V.) panamensis* was more common in the Pacific region. *L. (V.) panamensis* exhibited a broader geographic distribution than *L. (V.) braziliensis* (Perez-Franco et al. 2016; Patino et al. 2017; Correa-Cárdenas et al. 2020). Ecological conditions, vector species distribution, and the movement of both human hosts and animal reservoirs likely influence these patterns.

In this study, both geographic location and *Leishmania* species, particularly *L. (V.) braziliensis*, were significantly associated with TF. Most cases of *L. (V.) braziliensis* infection and treatment failure were concentrated in the Orinoco and Amazon regions, which is consistent with previous findings (Fernández et al. 2014). These variables should be considered essential determinants when choosing the optimal therapeutic strategy for managing CL in endemic areas.

Susceptibility test of isolated *Leishmania* strains to meglumine antimoniate

To assess whether drug resistance contributed to TF, the susceptibility of isolated *Leishmania* strains to MA was evaluated through EC_{50} measurements. Of the 44 clinical samples collected, only 13 produced viable cultures five *L. (V.) braziliensis* and eight *L. (V.) panamensis* none of which showed in vitro MA resistance. These results suggest that TF was more likely driven by clinical or epidemiological factors rather than intrinsic parasite resistance. Other unassessed contributors may include host immune response, drug pharmacokinetics, and the presence of *Leishmania RNA Virus 1* (LRV-1), previously associated with more severe disease and poor therapeutic outcomes (Adaui et al. 2016; Ponte-Sucre et al. 2017; Conceição-Silva et al. 2018).

Recently, Gómez et al. (2024) identified host immune mechanisms linked to treatment failure using sequential transcriptomic profiling of monocytes, neutrophils, and eosinophils. Persistent activation of the type I IFN pathway characterised patients who failed therapy, and a nine gene expression score accurately predicted treatment outcome. These findings support the potential of host based biomarkers and targeted therapies to improve treatment efficacy in cutaneous leishmaniasis.

This study has several limitations. The relatively small sample size, particularly the number of TF cases, may have limited the statistical power to detect associations or include variables such as lymphadenopathy in multivariate models. Due to logistical constraints, only a subset of isolates was cultured for in vitro susceptibility testing, restricting a full assessment of drug resistance. Moreover, host immune responses, treatment adherence, and genetic factors affecting drug metabolism were not evaluated. Future studies integrating immunological, pharmacological, and genomic analyses are needed to elucidate the multifactorial nature of TF and to develop more individualised therapeutic strategies. Molecular characterisation of clinical isolates also remains essential to identify resistance mechanisms.

Although military operations have recently decreased in some endemic regions, leishmaniasis remains a major public health concern in Colombia, particularly given the rising rates of therapeutic failure. In military settings, where exposure to endemic areas is inherent to operational duties, the risk of infection remains high. Our findings underscore the importance of addressing key risk factors such as prior treatment, lesion characteristics, geographic exposure, and parasite species and of implementing follow-up protocols and predictive tools to guide clinical decision-making. Continued investment in research, surveillance, and clinical monitoring is vital to strengthen responses to CL at national and regional levels.

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Author contributions Paula Ximena Pavia : Conceptualisation, Methodology, Validation, Investigation, Formal Analysis, Resources, Original Draft Writing, Review & Editing, Visualisation, Supervision, Project Administration, Funding Acquisition. Luz H. Patiño : Methodology, Original Draft, Writing Review. Claudia Méndez : Conceptualisation, Resources, Writing Review, Supervision, Project Administration and Funding Acquisition. Yanira Romero : Methodology and resources. Maria Clara Duque : Investigation, Resources, and Acquisition of Funding Claudia Cruz : Methodology, validation, investigation, and resources. Juan David Ramírez : Conceptualisation, Methodology, Validation, Investigation, Original Draft Writing, Review & Editing, Visualisation.

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Data availability The data supporting the findings of this study are available from the corresponding author upon reasonable request. All sequences obtained were deposited at the European Nucleotide Archive of the European Bioinformatics Institute under project accession numbers PRJEB77615 (ERZ24812769) for the HSP70 gene and PRJEB77616 (ERZ24812770) for the MPI gene.

Code availability Not applicable.

Declarations

Ethical approval This study was approved by the Research Committee (Project code 2015-048) and the Ethics Committee of the Central Military Hospital (Approval No. 35420; date: 26/11/2015).

Consent to participate Each patient provided written informed consent before inclusion using a form approved by the Ethics Committee of the Central Military Hospital (Approval No. 35420; date: 26/11/2015).

Competing interests The authors declare no competing interests.

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