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Review Article

Leishmania antimony resistance/susceptibility in Algerian foci

Abstract

Algeria is one of the most endemic countries for cutaneous and visceral forms of leishmaniosis. Strikingly, with more than 21,000 annual cases of cutaneous leishmaniosis recorded in 2010, the disease has a major public health impact. For all forms of leishmaniosis, the first line treatment relies on antimonial containing drug i.e., Glucantime®, developed during the 1950's. As early as 1986, antimonial treatment failure was reported during cutaneous leishmaniosis treatment. Linked to this therapeutic failure, *Leishmania* strains displaying a low susceptibility towards antimonials were isolated. Nevertheless, in Algeria, antimonial formulations still remain the first line drug for all clinical forms of leishmaniosis. Therefore, an urgent need of knowledge on the baseline antimony susceptibility status of parasites strains in Algeria is required. These pieces of knowledge will shed light not only on the prevalence of antimony resistance in this area but also on underlying factors triggering this drug resistance in natural populations. Here, we performed a review of the literature on what is known about epidemiology, treatment failure, and antimony-resistance, in Algeria. We bring information on underlying mechanisms acting in antimony resistant parasites and discuss their potential to be used for diagnostic purpose. This analysis will help to set up protocols aiming at detecting antimony resistant strains in Algeria and to test the risk of transmission, two steps that are essential to define public health policy in Algeria.

peruviana, *Leishmania (Vianna) guyanensis*, *Leishmania (Vianna) panamensis* and others), mucocutaneous leishmaniosis caused by *Leishmania (Vianna) braziliensis*, diffuse cutaneous leishmaniosis caused by *Leishmania amazonensis* and *Leishmania mexicana* and ZVL caused by *Leishmania infantum*, are all highly endemic. In the Indian subcontinent, anthroponotic visceral leishmaniosis (AVL) caused by *Leishmania donovani* poses a major health problem. In several regions of the world, the incidence of leishmaniosis outbreaks has been associated with urbanization, travel, climatic change and social conflicts [2,3].

In the absence of effective vaccines, the only feasible way to treat and control leishmaniosis is through the use of affordable medications. The current chemotherapeutic arsenal consists of molecules developed in the 1950s', including pentavalent antimony (Sb(V)) compounds (e.g., Pentostam®, Glucantime®), pentamidine and various formulations of the antifungal Amphotericin B and, more recently, miltefosine [4-7]. The choice of the chemotherapeutic protocol is frequently dictated by economic considerations instead of being based on clinical or biological indications. Therefore, antimonial containing drugs, one of the most affordable molecules, are in clinical use to treat all forms of leishmaniosis in most part of the world [8]. Therapeutic failure during antimony treatment remains a well-known problem, but antimony resistance is not the only factor responsible for the therapeutic failure. Indeed,

Introduction

Leishmania sp. are causative protozoal agents of various forms of leishmaniosis, which is a significant cause of morbidity and mortality in more than 98 countries and territories [1]. The clinical manifestations of leishmaniosis range from various cutaneous forms to a fatal visceral form. In the Mediterranean Basin, anthroponotic cutaneous leishmaniosis (ACL) caused by *Leishmania tropica*, zoonotic cutaneous leishmaniosis (ZCL) caused by *Leishmania major* or, less frequently *Leishmania infantum* and zoonotic visceral leishmaniosis (ZVL) caused by *L. infantum* are endemic. Visceral leishmaniosis is widely distributed around the Mediterranean Basin, unlike ZCL and ACL which are more restricted to the southern and oriental regions. In South America, ZCL caused by various *Leishmania* species (e.g., *Leishmania mexicana*, *Leishmania (Vianna)*

factors that are linked to the host (e.g., immunosuppression or malnutrition), the drug itself (e.g., drug batch or counterfeit drugs), the Leishmania species, the practitioner or the patient (e.g., incomplete treatment follow-up) will also play a role in parasite drug resistance and treatment failure [4,9,10]. In most parts of the world, the frequency of parasite antimony resistance linked to treatment failure is unknown [4,9,10]. This information should be crucial to address the risk of selection and transmission of drug-resistant parasites, particularly in areas where antimony is the only chemotherapeutic alternative. Here we review the current epidemiological situation of leishmanioses in Algeria, address available tools to diagnose antimony resistance and, then focus on ongoing researches about antimony resistance in this area.

Update on past and current epidemiological data

The first case description of leishmaniosis in Algeria was carried out by the Sergent brothers almost a century ago [11].

Human visceral and cutaneous leishmanioses

In Algeria, three distinct clinical forms of leishmaniosis are encountered. In the Sahara and the highland regions, the zoonotic form of leishmaniosis, caused by *L. major* (ZCL), is endemic (Figure 1). Two variants of *L. major*, zymodeme MON-25, and zymodeme MON-269 are reported to be present in Algeria [12,13]. The insect vector for *L. major* is *Phlebotomus papatasi* [14], and the wild rodents *Psammomys obesus* and *Meriones shawi* are the reservoir hosts of the parasite [15,16]. Moreover, natural infection of hedgehogs with *L. major* was reported [17].

In the central part of the country and particularly in the oasis of Ghardaia, the chronic form of cutaneous leishmaniosis is due to *L. killicki*. *L. killicki* belongs to the *L. tropica* complex and generally occurs in sympatry with *L. major* [18,19]. The annual incidence of this peculiar form of cutaneous leishmaniosis is

estimated to be less than 100 cases per year, in Algeria. The proven vector for *L. killicki* is *Phlebotomus sergenti* [20], and the suspected reservoir host is *Massoutiera mzabi*, a rodent close to *Ctenodactylus gundi* that has been found naturally infected with *L. killicki* in Tunisia [21]. In the northern part of the country, a zoonotic cutaneous form caused by *L. infantum* is sporadic. The proven vector is *Phlebotomus perfiliewi* [22], and the variant *L. infantum* MON-24 [23], responsible for this cutaneous form, is present in dogs, the animal reservoir of this species. The visceral form of leishmanioses caused by *L. infantum* occurs mainly in the northern part of the country. The active visceral leishmaniosis foci are located in the region of Kabylie in the north of the country as well in the east in the departments of Jijel and Constantine. Some cases are also reported in the south, in Tassili N'ajjer and the Hoggar mountains in Illizi and Tamanrasset respectively [24]. The proven vector of *L. infantum* in Algeria is *Phlebotomus perniciosus* and canids, dogs and jackals are the main reservoirs [12,25].

Currently, an emerging focus of cutaneous leishmaniosis in the northern part of Algeria is reported [26]. Parasitological investigations carried out from 2004 to 2010 have confirmed *L. major* (zymodeme MON-25) to be the etiological agent of this outbreak, with *Psammomys obesus* as the main reservoir of the parasite. An entomological survey performed in 2009 pinpointed the predominance of two species of sandfly in this area: *Phlebotomus papatasi* and *P. perniciosus*. Therefore, cutaneous leishmaniosis caused by *L. major* is no longer confined to the central and southern part of Algeria but has spread from the arid zones towards the semi-arid areas, particularly in the Soummam valley. Climate changes and desertification observed in the steppe area of northern Sahara could have played a role in the extension of the disease [27].

Canine leishmanioses in Algeria

Since 1908 it is recognized that the dog plays a major role in the life-cycle of *Leishmania infantum* [28]. Various sandfly species belonging to the genus of *Phlebotomus* act as vectors of the parasite [29]. Canine leishmaniosis (CanL) is a cosmopolitan disease with a worldwide distribution, mainly in tropical areas, but also in the temperate zones of North Africa, Europe, and Asia [30]. The Mediterranean basin is particularly affected and the disease is reported in almost all the countries bordering the Mediterranean, with relatively variable prevalence. In Algeria, the general prevalence of CanL has been under 11% for many years, it has recently increased to 25.1% in 2006 [31]. This increase in the prevalence of canine infection was followed by an increase in human leishmaniosis cases. In the region of Algiers for example, 12.2% more cases of human visceral and cutaneous leishmaniosis were recorded between 1990 and 1997 [32,33]. Until the 1980s', the prevalence of infection amongst dogs of Algiers was always below 10%. It is only in the last decade that the prevalence markedly increased, to reach 30% in the 1990s' and 20%-25% in the 2000s'. This change may be mainly explained by the concurrent and dramatic environmental changes that have occurred in Algeria, particularly the uncontrolled urbanization that took place in recent years. This new urbanization has led to a proliferation of

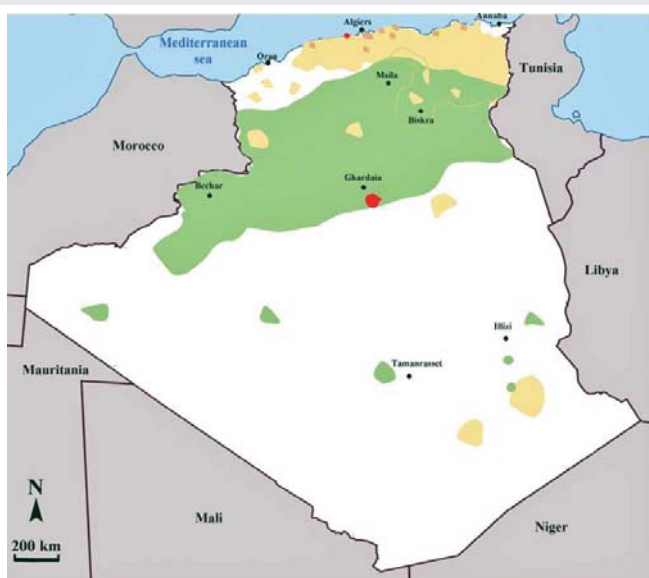


Figure 1: Distribution of cutaneous and visceral leishmaniosis in Algeria. (●) *L. major* cutaneous leishmaniosis, (●) *L. infantum* visceral leishmaniosis, (●) *L. infantum* cutaneous leishmaniosis, (●) *L. killicki* cutaneous leishmaniosis.

dogs around the houses and the removal of natural vegetation. That has possibly driven vectors to invade the peridomestic and intra-domiciliary habitats. Six zymodemes were identified in Algeria: MON-1, MON-24 and MON-281 in Algiers, MON-34, and MON-77 in Grande Kabylie [23,31,34-36]. An exhaustive view of the *Leishmania infantum* zymodemes isolated and characterized in human and dogs, around the Mediterranean basin are presented in figure 2.

Treatment, chemotherapeutic failure and drug-resistance in Algeria

Pentavalent antimonial compounds remain the first line treatment against both cutaneous and visceral forms of leishmaniasis in North Africa. In Algeria, care for leishmaniasis is provided for free by health structures. The diagnosis of CL takes place at health center level but visceral forms are diagnosed in hospitals. Meglumine antimoniate and conventional amphotericin B are included in the National Essential Drug List. The protocol recommended by the Ministry of Health, for the treatment of all forms of cutaneous leishmaniasis consists in the administration of 20 mg/kg/day Glucantime® (pentavalent antimony (Sb(V)) for 15 consecutive days, by intramuscular injection, when multiple lesions are observed, or if the lesion is located on the face. For single lesion, the Ministry of Health recommends the intradermic (intralesional) administration 1.5 to 2 ml of Glucantime® twice per week for 4 weeks. Hydrogen peroxide (H₂O₂ 10 vol) or cryotherapy is also used to treat cutaneous lesions as alternative treatments.

For VL, intramuscular injection of Glucantime® (20 mg Sb(V)/kg/day) for 28 days is recommended by the ministry of health, following the protocol recommended by the World Health Organization. In case of unresponsiveness, intravenous perfusion of amphotericin B (Fungizone®) in a dose of 1 mg/kg/day for 15 days is recommended.

In Algeria, unresponsiveness to Sb(V) to the treatment of

zoonotic cutaneous leishmaniasis is well documented in the focus of M'sila. In 1986 a survey carried out on ninety-seven children who received 60 mg/kg/day of meglumine antimoniate for 15 days, reported no significant response compared to those receiving placebo. *In vitro* tests performed on intracellular amastigotes confirmed that all the strains of *L. major* isolated from these children displayed a low susceptibility towards Sb (V) containing drugs in the form of Glucantime® [37]. More recently, 9% of unresponsiveness to antimonial treatment was recorded in children with visceral leishmaniasis [38,39].

For the veterinarian practitioners, treatment of canine leishmaniasis remains difficult, given the complex pathogenesis of the disease associated with pleiotropic clinical signs [40]. All antileishmanial compounds in use to treat dogs can lead to temporary or permanent remission of clinical signs but no established protocol is effective to eliminate the infection. While therapeutic protocols have evolved the drugs used for treatment haven't changed. In case of a confirmed diagnosis, the practitioner has firstly to worry about the risks of transmission to man and then about chances of curing of the animal [41]. Infected dogs are often treated with the same drug used for humans. The treatment with antimonials is often long and costly and the toxicity of antimony causes serious side effects [42,43]. Currently, the association of meglumine antimoniate with allopurinol is used [43,44]. In Algeria, dog suffering of leishmaniasis is usually not treated. Therefore, there are no available data on treatment efficiency. So as in other countries endemic for the leishmaniasis, veterinarian practitioners euthanize dogs diagnosed as positive in order to prevent the spread of the disease to humans.

In the occidental part of the Mediterranean basin where dogs are commonly treated with meglumine antimoniate, the occurrence of resistant strains to antimony is documented [45]. We recently highlighted the occurrence of antimony-resistance in strains belonging to the *L. infantum* MON-281 [46] and further probed their potential of being transmitted by sandflies [47]. We also checked for cross-resistance with other molecules in clinical use to treat the visceral leishmaniasis [2,46,48]. To clarify the risk of transmission of antimony-resistant strains in Algeria, it will be crucial to determine the prevalence of the peculiar zymodeme MON-281 circulating in the canine population. To shed light on the underlying factor that may act to select for antimony resistance, we have begun to assess and compare the *in vitro* antimony susceptibility status of strains originating from two geographic areas, differing in their history of antimony pressure. A sample of 48 and 20 strains isolated from humans, dogs or sandflies during the last 30 years, in Algeria and France respectively, was selected in the Biobank of the Pasteur institute of Algiers and the National Reference Center for Leishmania in Montpellier. The antimony susceptibility status of strains was tested on promastigotes. Interestingly, *L. infantum* strains isolated in Algeria were found to be significantly less susceptible toward antimony than strains isolated in France ($p < 0.001$, unpaired t-test) (Figure 3) (Eddaikra et al., unpublished results). The selection for antimony resistance was recently demonstrated to confer fitness advantages [49,50]. This finding will likely have

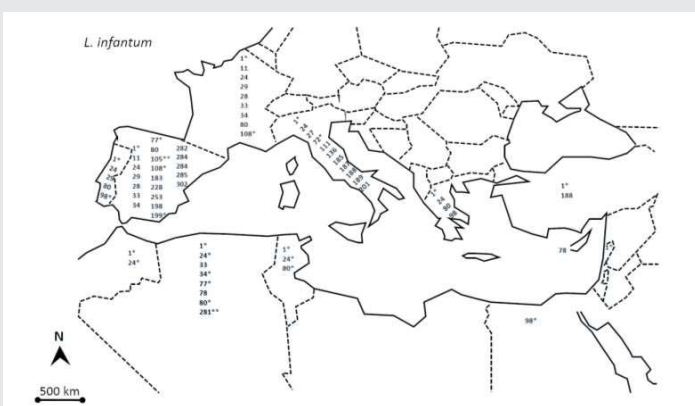


Figure 2: Distribution of *L. infantum* variants responsible for canine and human visceral leishmaniasis, around the Mediterranean basin. Between 1973 and 2010, numerous human and canine *L. infantum* isolates were collected in 15 countries excepted in Algeria, bordering the Mediterranean sea. Parasites were typed by multilocus enzyme electrophoresis (MLEE), according to the protocol previously described by Rioux *et al.* (1990), using 15 enzymatic systems. The numbers represent the zymodemes. (*) Only 12 zymodemes out of 37 reported in human were found in dogs, namely MON-1, MON-11, MON-24, MON-27, MON-30, MON-34, MON-77, MON-98, MON-108, MON-267, MON-278 and MON-281. (**) only found in dog.

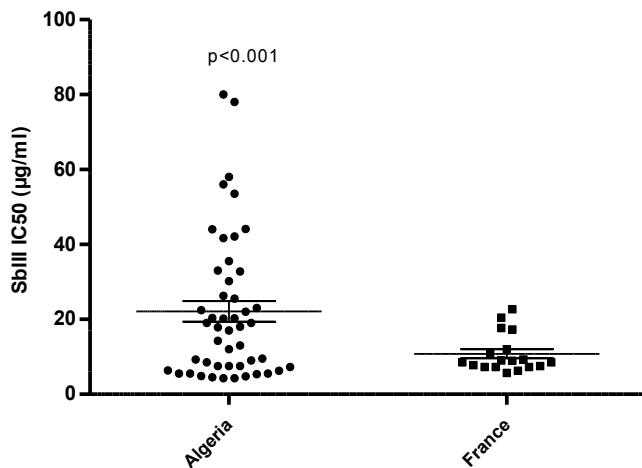


Figure 3: Antimony susceptibility status of *L. infantum* strains isolated in Algeria and France. Susceptibility (IC_{50}) toward trivalent antimony of *Leishmania infantum* MON1 promastigotes isolates sampled in Algeria and France.

to result in the prioritization of studies aimed at diagnosing antimony resistance in Leishmania.

Available tools to monitor parasite antimony susceptibility

Various hosts and antimony-derived molecules play a role in the antileishmanial activity of Sb (V) *in vivo*. Therefore, ideally, to reflect the *in vivo* activity of antimonials, the *in vitro* tests must monitor the antileishmanial activity of compounds playing a role in the killing pathway of Sb (V). In addition, the origin of the antimonial batch has to be taken into account, because commercially available Sb (V) solutions are not pure [4,51]. *In fine* in all cases, the IC_{50} determined *in vitro* must always be compared with clinical observations. In most laboratories, the screening for leishmanicidal compounds is carried out with *Leishmania* promastigotes. However, the best approach is to test leishmanicidal compounds toward amastigotes residing in macrophages.

Cellular models for *in vitro* testing antimonial susceptibility

Obviously, the intracellular amastigotes represent the ideal model because both the indirect activity, through the host cell, and the direct one on the parasite can be assessed. Unfortunately, methods that involve intracellular amastigotes are labor intensive, difficult to standardize and time-consuming. In addition, the results are dependent upon the nature of the host cell used to grow *Leishmania* amastigotes [52–54]. Duplex quantitative reverse-transcriptase PCR (qRT-PCR), aimed at assessing drug activity against *Leishmania* intracellular amastigotes and their host cells can be performed. The assay simultaneously quantifies *Leishmania* 18S ribosomal RNA and the human β 2-microglobulin (β -2M) mRNA, used for monitoring drug cytotoxicity and test performance [55]. Nevertheless, this approach is time-consuming, cost prohibitive and not amenable to large-scale analysis. The development of reporter gene technologies has enabled the quantification of *Leishmania* parasites in host cells and whole mammalian hosts [56–59]. These technologies have been tested

to determine the drug susceptibility of field isolates [60,61]. However, the time required for transfection and the selection of recombinant parasites certainly affect the composition of the isolates in mixed *Leishmania* infections [62]. But the lack of consensus in the standardization of this methodology makes interpretation of potential intra- and interspecific variations in antimony susceptibility difficult. In fact, even if this reporter-based method takes into account all the factors that are involved in intracellular Sb (V) antileishmanial activity, its versatility and cost make its application very impractical for large-scale analysis of parasite antimony susceptibility and resistance surveys. More recently high content imaging proved to be useful for antileishmanial agent discovery [63], since no reporter gene is used to monitor the intracellular parasite multiplication. This technology should represent the future for antimonial susceptibility phenotyping of natural *Leishmania* populations.

A large number of *Leishmania* species can be grown *in vitro* as amastigotes under axenic conditions [64,65]. The determination of drug activity is simple, typically inexpensive and does not require host cells, which makes standardization easier. Various studies showed a similarity of protein contents in axenic amastigotes and intracellular ones. In addition, axenic amastigotes seem much more infective and grow more easily than those isolated from macrophages-derived cells. Axenic macrophages can be also differentiated *in vitro* directly from the promastigote stage. However, as for the reporter gene technology, the duration of the differentiation, the capacity of strains to be differentiated and their representation in the initial isolate to be tested may select strains pre-adapted to the differentiation into amastigotes and consequently modify the composition of isolates in mixed infections. Indeed, not all *Leishmania* species or strains are amenable to being cultured in axenic conditions. Currently, no extensive studies using axenic amastigote cultures have been performed to evaluate the antimony susceptibility of field isolates.

The usefulness of promastigotes to ascertain the antimony susceptibility status of *Leishmania* field isolates has been debated. The trivalent form of antimony (Sb (III)) is used to test the susceptibility of promastigotes because this stage is weakly susceptible to the pentavalent antimony. Unfortunately, the susceptibility of promastigotes to trivalent forms of antimony does not always reflect the sensitivity of intramacrophagic amastigotes to pentavalent antimonial formulations [66,67]. Nevertheless, various experimental reports that used *L. tropica*, *L. infantum* or *L. donovani* support the notion that Sb (III) susceptibility of promastigotes partially reflects the intrinsic Sb (V) susceptibility of amastigotes within macrophages [68–70]. Because the host cell is omitted in tests that use promastigotes, we hypothesized that the discrepancies observed between experiments might reflect the differential role of the host-derived microbicidal factors involved in the Sb (V)-induced intracellular killing. Most of these drug or host-derived factors are currently identified, but tests aimed at simultaneously ascertaining the intrinsic susceptibility of promastigotes to antimonials might be more representative.

Animal models for *in vivo* antimonial susceptibility testing

Animal models have been largely used to study the biological and pathological features specific to cutaneous and visceral leishmaniasis, even that none can reproduce the various syndromes encountered in humans. Current animal models aim at investigating (i) host–parasite interactions by the way of pathogenesis or biochemical and biological changes, (ii) *in vivo* maintenance of parasites and (iii) clinical evaluation of drug candidates.

The principal problem of using animal models to explore antimony activity is the route of administration and the distribution to different sites of infection. Several animal species served as experimental hosts especially for CL: BALB/c mice and Syrian golden hamster, dogs and monkeys [71,72]. The choice of convenient animal models depends on *Leishmania* species under study. For instance, the most suitable animal models are C57BL/6 mice (or vervet-monkeys, or rhesus-monkeys) for *L. major* and CsS-16 mice for *L. tropica*. CBA mice are convenient to study physiopathological effects caused by the infection with *L. amazonensis* and CBA and golden hamsters or rhesus-monkeys can be used for *L. braziliensis*⁷¹. We have recently established a murine model for *Leishmania killicki* cutaneous infections and probed its suitability for pharmacological purposes [73]. This new model will now enable us to investigate the *in vivo* behavior of this emerging pathogen, under antimony *in vivo* antimony pressure.

Biomarkers for antimony resistance

The search for resistance biomarkers is based on the in-depth understanding of the antileishmanial mode of action of antimony (Figure 4) and resistance associated with *in vitro* selection [74]. Studies on the intracellular mode of action of Sb(III) demonstrates that thiol buffer capacity of *Leishmania* is deeply affected by Sb(III), through the inhibition of the trypanothione reductase [75], and via a decrease in the intracellular thiol content through an efflux mechanism yet unidentified [76]. These two mechanisms combine to profoundly compromise the thiol redox potential in drug-sensitive parasites and lead ultimately to the accumulation of reactive oxygen species ROS [76,77].

Many of the specific genes associated with resistance to antimony have been discovered. These mechanisms generally involved:

The limiting antimony entry into the cell. The entry of Sb(III) occurs through an AQP1 transporter [78], but the route of entry of Sb(V) is currently not identified.

The increase of the target expression. Sb(III) inhibits the Trypanothione reductase activity leading to an accumulation of the reduced form of trypanothione. Overexpression of the Trypanothione reductase has been characterized in most of the antimony-resistant field isolates [79], with some exceptions [80].

The decrease of the reduction of the prodrug form of Sb, Sb(V) to the active form Sb(III). This function is assumed by

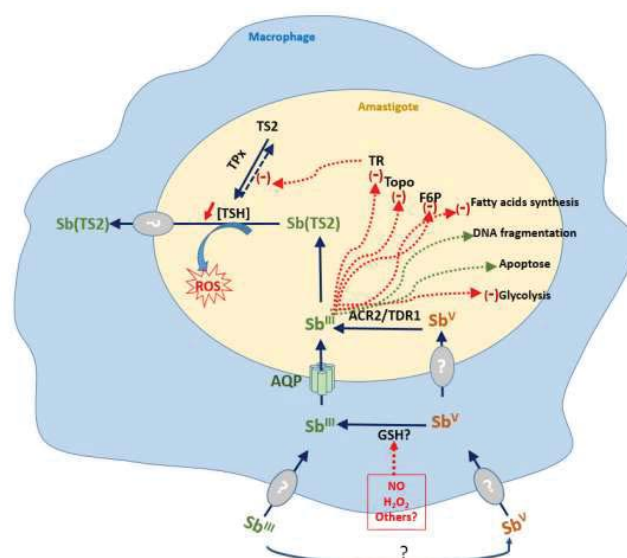


Figure 4: Schematic representation of the proposed mode of action of Sb(V) containing drugs. The trivalent form of antimony (Sb(III)) comes from the reduction of the pentavalent form Sb(V) and constitutes the active form of antimony towards *Leishmania*. In the host cell, the Host-derived molecules also play a role in the antileishmanial activity of Sb(V) *in vivo*. Both species of antimony are able to induce the generation of NO and H₂O₂ that are harmful for intracellular parasites. The entry of Sb(V) into the parasite occurs by an unknown transporter, and entry of Sb(III) through AQP1. Within the parasite, Sb(V) is reduced into Sb(III) by the action of the reductases, ACR2 (an homolog of the yeast arsenate reductase) and TDR1 (Thiol Dependent Reductase). Beforehand Sb(V) can be also reduced by Glutathione in the host cell. Sb(III) inhibit various enzymes: the topoisomerase (Topo), the fructose-6 phosphatase (F6P) and the trypanothione reductase (TR). It interferes with the antioxidative defense of the parasite through the inhibition of the trypanothione reductase and the formation of Sb(III)-TSH complex that is actively exported. These actions led to a drastic reduction in TSH and concomitantly to an accumulation of reactive oxydant species (ROS) toxic for the parasite. Other abbreviations: TPx, Tryparedoxin, TSH, reduced trypanothione; TS2, oxidized trypanothione; Sb(TS)2, conjugate of Sb(III) with trypanothione.

two reductases, TDR1, a thiol-dependent reductase, belonging to the Glutathione S-transferase family that shares homologies with the *T. cruzi* Tc52 protein [81], and LmACR2 that shares homologies with arsenate reductases [82]. Nevertheless, variations in reductase capacity of field *Leishmania* isolates resistant to antimony have never been investigated.

The conjugation of the drug with thiol-containing molecules and sequestration or efflux of the thiol conjugated drug. In all Kinetoplastidae, the major low molecular mass thiol is trypanothione in contrast with most other prokaryotic and eukaryotic cells, which utilize glutathione to maintain the intracellular thiol redox potential and preserve cells from harmful attacks of oxidative compounds. Trypanothione is a glutathione–spermidine conjugate that is formed following several enzymatic steps, via the synthesis of reduced glutathione (GSH) and its subsequent conjugation to spermidine. Steps in the synthesis of spermidine involve ornithine decarboxylase (ODC) and spermidine synthase. The first step in the biosynthesis of GSH is catalyzed by the γ -glutamylcysteine synthetase (γ -GCS) and the glutathione synthase. In the following step, two enzymes specific to trypanosome are involved in the pathway. First, the glutathionylspermidine synthase ligates reduced glutathione (GSH) to spermidine in a conjugate, the glutathionylspermidine. A second Kinetoplastidae-specific

ligase, the trypanothione synthase conjugates a second GSH to glutathionylspermidine to produce trypanothione. The pool of reduced trypanothione is maintained by the trypanothione reductase (TR) that ensures the thiol redox potential. Consequently, the overexpression of genes that leads to an increase in the intracellular level of reduced trypanothione (T (SH)₂), involves the formation of the Sb–trypanothione conjugate (Sb(TS₂)) and its sequestration within a vacuole by the intracellular ABC transporter MRPA [83], or its efflux from the parasite. In that way, the conjugation of Sb with trypanothione induces antimony resistance by using up free Sb (III) [81], within the amastigote. However, enzyme that conjugate Sb with trypanothione or other molecules containing thiols, is unknown.

Mutations associated with resistance represent molecular signatures largely used in drug resistance surveillance for other parasitic diseases. For instance, single nucleotide polymorphisms (SNPs) associated with *Plasmodium* susceptibility to drugs are often good indicators of the clinical drug resistance [84]. Unfortunately, no SNP have been proved to be indicative of antimony resistance in field isolates. But another main mechanism of resistance evidenced in *Leishmania* consists in genomic rearrangements, which lead to the amplification of resistance genes through homologous recombination between repeated sequences [85]. Amplified genes known to be involved in antimony susceptibility should, therefore, represent good biomarkers for addressing antimony resistance [86].

If some of the above mentioned mechanisms are effectively encountered in resistant strains from the field, any rule could be applied mainly because of the presence of additional unrelated or less specific mechanisms [87–90], or because of other yet unidentified mechanisms of resistance. Nevertheless, significant DNA amplification and/or higher RNA expression occur in a majority of strains having an antimony resistant [91].

Recent metabolomic approaches have been used to investigate parasites displaying different susceptibility to antimony. Interestingly, a hierarchical clustering approach (antimony resistant and susceptible) revealed differences in the metabolite abundance for the drug-resistant and -sensitive clones [92]. However, in this study the sample size was not large enough to conclusively identify drug-resistant parasites. The extensive procedures involved in culturing the parasites, the high cost, the large amount of time required and the need for highly skilled technicians to perform the metabolomic analyses limit their usefulness for antimony susceptibility surveys. But metabolomic studies might provide information on new metabolic markers for monitoring antimony resistance.

Conclusions

In Algeria, Sb (V) resistant *L. major* and *L. infantum* have been isolated from unresponsive patients and animal reservoirs [37,46]. Therefore, the emergence of such resistant parasites stressed questions about factors that are acting to select them but also required more knowledge on the risk of their transmission. In order to compare the relative fitness

of antimony-resistant and susceptible parasites, it would be interesting to survey the prevalence of both phenotypes over a large period of time. We think that it would be an adequate approach to assess the relevant natural fitness of both phenotypes. The former is essential to study each of the fitness components in more details, in order to understand why the relative fitness between phenotypes differs, (e.g. due to differences in survival, reproduction or transmission [49,50]. These studies are of importance for public health policy.

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