Leishmaniasis is a disease complex caused by 17 different species of protozoan parasites belonging to the genus *Leishmania*. The parasites are transmitted between mammalian hosts by phlebotomine sandflies. Leishmaniasis has a worldwide distribution with important foci of infection in Central and South America, southern Europe, North and East Africa, the Middle East, and the Indian subcontinent. Most forms of leishmaniasis are zoonotic, human beings affected only secondarily, but two species of *Leishmania* can maintain arthropoetic, human-human cycle. These are *L. donovani*, the species responsible for VL in the Indian subcontinent and East Africa, and *L. tropica*, which is responsible for CL in the old World.

VL, also known as kala azar is a protozoan systemic infection, which is almost always fatal if left untreated. This illness is endemic in several tropical and subtropical regions and in the Mediterranean basin. The estimated annual global burden of VL is 500,000 new cases and more than 50,000 deaths, of which 90% occur just in five countries—India, Bangladesh, Nepal, Sudan, and Brazil. VL is transmitted through hematophagous sandflies and is caused by *L. donovani* in the Indian subcontinent, Asia, and Africa, *L. infantum* in the Mediterranean basin, and *L. chagasi* in South America. After an incubation period of several months, typical VL manifests with intermittent fever, weight loss, massive hepatosplenomegaly, and progressive deterioration of the host; hemorrhages and edemas may develop late in the course. Leishmaniasis was selected by the World Health Organization for elimination by 2015, along with other neglected tropical diseases. Since there is no antileishmanial vaccine in clinical use, control of VL relies almost exclusively on chemotherapy.

For almost seven decades pentavalent antimonials constituted the standard antileishmanial treatment worldwide, however the last 15 years their clinical value was jeopardized due to the widespread emergence of resistance to these agents in Bihar, India, where half of VL cases occur globally. The last decade novel formulations of conventional antileishmanials as well as new drugs, including the oral agent miltefosine, became available or are under investigation. In practice, however, their wide use in poor countries is hampered mainly due to high costs and also due to concerns of toxicity and emergence of resistance. In response to concerns about preserving the currently available antileishmanials, especially in regions with anthroponotic parasite transmission, there is growing interest on combination regimens. This thesis will focus on the factors that cause variation in response to antileishmanial chemotherapy, evaluate the problems and mechanism associated with clinical resistance, and consider how a system for monitoring and surveillance might be implemented with associated implications for research.

Quest for a simple marker that specifies difference in responsiveness of *Leishmania donovani*...
isolates to wide variety of antileishmanial drugs is of fundamental importance to provide appropriate treatment to the patients. My first chapter deals with the speculation of specific markers to distinguish antimony resistant phenotypes from the sensitive ones. In the first section of this chapter, a number of biochemical parameters were studied to find out the difference, if any, among the recent *Leishmania donovani* isolates, and in this way assign the parasites as sensitive or resistant to antileishmanial drugs. The extent of resistivity to a particular drug among the field isolates has been assessed by the degree of clearance of amastigotes from infected macrophages (Mφ) represented by the EC$_{50}$ values of the four drugs Sodium stibogluconate (SSG), Miltefosine (Mil), Amphotericin B (AmB) and Paromomycine (Paro). The direct action of Mil against the promastigotes of the clinical isolates was also studied. The resulting half maximal effective concentration EC$_{50}$ values were compared with those of known sensitive isolates. The intracellular level of thiol metabolizing enzymes and surface expression of glycoconjugates (N-acetyl-D-galactosaminyl residue) were also compared in sensitive and resistant isolates. The statuses of surface ABC transporters (Multidrug resistant protein (MRP1) or P-glycoprotein (P-gp) responsible for antimony efflux from host and subsequent drug resistance in intracellular parasites) of the host cells upon their interaction with either resistant or sensitive isolates were also studied. The results reinforced the notion that resistant parasites have undergone a number of biochemical changes as part of their adaptation to ensure their survival in the host. This chapter provide informations that might help to design new tools for diagnosis of patients infected with antimony unresponsive isolates.

In order to combat the pressing problem of drug resistance, dissecting the metabolic pathways of antimony unresponsive and sensitive parasites are essential. Designing a drug which perturbs or affects the metabolic pathway of the resistant parasites might prove to be an efficient mean to eradicate the problem of drug resistance in field. The SSG resistant parasites are known to show more aggressive infection as compared to the sensitive ones in infected patients. The reason for such difference in virulence is known to be due to higher number of metacyclics in resistant parasites as compared to sensitive. In the second section of this chapter, such observation was established with a different set of clinical isolates. Our result correlated the report; therefore, we probed the possible reason for such a difference in growth rate. The differences in the rate of cell cycle, oxygen consumption during respiration, their intracellular ATP content were estimated and compared between the resistant and sensitive isolates. The difference in growth rate led us to speculate the relative rate of glycolysis and pentose phosphate pathway in these isolates. Overall, this work provided an understanding regarding the biochemical difference in the Glycolytic and Pentose phosphate pathway in antimony resistant and sensitive clinical isolates thereby providing a platform for designing appropriate drug targets and drugs to solve the problem of antimony resistance.
The differences in the biochemical parameters between these isolates were not sufficient to understand their disease pathogenesis and mode of interaction with the host. Therefore, the molecular mechanism of resistant parasite driven upregulation of MDR1 in infected M\(\phi\) has been investigated in the second chapter. Both promastigote and amastigote form of resistant but not sensitive parasite express a unique glycan with N-acetylgalactosamine as terminal sugar, removal of which enhanced the sensitivity towards antimonials. These terminal sugars are responsible for IL-10 overexpression in host cells. We traced the complex pathway in which we show the mechanism by which the parasite surface glycan and resulting intracellular IL-10 overexpression lead to MDR1 upregulation in M\(\phi\)s. This result indicates that the resistant parasites, as opposed to the sensitive ones, differentially interacts with host cells and gives rise to significant differences in the outcome of pathogenesis.

Several works are in progress to combat drug resistance in Leishmaniasis by targeting host cell ABC transporters. However, the unique method by which the antimony resistant parasites establish and show aggressive infection is still in its infancy. Another major feature in the mode of interaction with the host cells at early time point post infection is the ability of resistant isolates to induce host cell autophagy. Apart from parasite entry, proper establishment and exploitation of the host cell systems, the parasites need to disseminate in order to infect fresh set of cells and continue this cycle. For this, the parasites need to egress the exploited host cells without triggering any major immune response, to infect new cells of the visceral organs of the host. My last chapter deals with the mechanism of antimony resistant parasite induced transient autophagy in host cell followed by apoptosis which leads to parasite egress. Autophagy, a major cellular pathway for the degradation of cytoplasmic macromolecules and organelles, is crucial for cell survival in response to starvation and for preventing intracellular accumulation of abnormal protein aggregates. Usually autophagy acts as an innate immune defense mechanism by entrapping invading pathogens and targeting the resulting vesicles for fusion with lysosomes. However, intracellular antimony resistant *Leishmania* have evolved distinct mechanisms to survive and multiply within membrane-bound compartments. This study traces the intricate pathway involving the mechanism by which antimony resistant parasite induces host cell autophagy at early time point post infection and the post transcriptional regulation leading to abrogation of autophagic pathway and triggering of apoptotic pathway at late time point post infection. The apoptotic pathway might lead to effective parasite egress and aid in further propagation. Developing means of selectively inhibiting autophagy in infected cells should therefore be viewed as a new window of opportunity in dealing with hard-to-eliminate intracellular drug resistant pathogens.