Mechanism of Resistance of Some Neglected Diseases

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Neglected diseases (NDs) are a group of diseases endemic in underdeveloped and developing countries and have been a global health problem, such as leishmaniasis, tuberculosis, cryptococcosis, leprosy among others. Drugs used in the clinic are toxic, they do not always result in a cure, and many parasites have shown resistance to them. In the last years, the incidence and prevalence rates of some Neglected Diseases have decreased in the world, opposite to the multi-drug-resistance (MDT) levels observed. Drug resistance in leprosy and tuberculosis becomes even more important because they’re very limited alternative drugs to MDT. Molecular studies on the mechanism of action of these drugs have elucidated the genetic basis of drug resistance in Mycobacterium tuberculosis and Mycobacterium leprae, respectively. C. neoformans has been shown to present different susceptibility profiles to antifungal drugs according to in vitro studies, although little reports of resistant cases have been described. C. neoformans resistance can be seen to azoles, especially fluconazole, to amphotericin B (AmB) and to 5-flucytosine (5FC). This chapter will summarize the main mechanisms of resistance of some neglected diseases such as leishmaniasis, leprosy, cryptococcosis and other.

Keywords Neglected diseases, leishmaniasis, leprosy, cryptococcosis, multi-drug-resistance.

1. Introduction

Neglected tropical diseases (NTDs) are a group of tropical diseases endemic in underdeveloped and developing countries and have been a global health problem. NTDs constitute one of the main challenges to medical science in the last century. Thus, the development of molecular technologies have greatly increased our knowledge of the evolution, transmission and pathogenicity of this diseases. Research conducted in different country has shown that host susceptibility to many infectious diseases has a genetic basis. Currently, with the advent of molecular epidemiology, much has been known about the virulence, evolution, as well as the mechanisms of resistance and susceptibility drugs. In general, treatment failure can occur due to issues associated to treatment (e.g. compliance, dosage, quality), parasite related (e.g. intrinsic or acquired drug resistance) or host-related (e.g. pharmacogenetics, immune response) factors [1]. This chapter will summarize the main of drug resistance mechanisms of some neglected diseases such as leishmaniasis, leprosy, tuberculosis, and fungal infections as cryptococcosis and candidiasis.

2. Tuberculosis - Drug Resistance Mechanism

Members of the M tuberculosis complex use several strategies to resist the action of antimicrobial agents; the highly hydrophobic cell wall, the drug efflux systems and inactivating enzymes are the most knowledge [2]. Resistance-associated point mutations, deletions or insertions have been described for all first-line drugs (isoniazid, rifampin, pyrazinamide, ethambutol, and streptomycin), and for several second-line drugs (ethionamide, fluoroquinolones, macrolides) [3].

Isoniazid (INH) is a pro-drug, requiring oxidative activation by the catalase-peroxidase enzyme KatG [4,5]. It appears to penetrate host cells readily and diffuses across the M. tuberculosis membrane [6, 7, 8]. The primary inhibitory action of INH in sensitive mycobacteria is on mycolic acid synthesis, although it has been reported to inhibit synthesis of nucleic acids, phospholipids, and NAD metabolism [9, 10, 11, 12].

The INH binds two intracellular targets: the fatty-acid enoyl-acyl carrier protein reductase (InhA) and a β-ketoacyl-ACP synthase (KasA), that are involved in synthesis of mycolic acids. Mutations have been found in the promoter regions, or less commonly in the genes that encode these proteins (inhA, acpM, and kasA). However, the role of kasA mutations in isoniazid resistance is presently unclear, because similar mutations were also found in isoniazid-susceptible isolates [13, 14].

The mechanism of action of rifampin (RIF) is the same described to M. leprae. More than 96% of the rifampin-resistant isolates of M. tuberculosis contain mutations in a well-defined, 81-bp (27-codon) central region of the gene encoding the beta subunit of RNA polymerase (rpoB) [15].

Pyrazinamide (PZA), likes INH is a pro-drug that needs activation by the enzyme pyrazinamidase (PZase) [16, 17]. PZA act against bacilli residing in acidified compartments of the lung, mainly in inflammatory sites of infection [18]. PZA enters tubercle bacilli passively and via an ATP-dependent transport system [19]. Intracellular accumulation of the drug occurs because of an inefficient efflux system in M. tuberculosis. The anti-tuberculosis activity of PZA has been attributed to disruption of the proton motive force required for essential membrane transport functions by PZA at acidic
expression and a 4- to 8-fold increase in MIC [50]. Subinhibitory concentrations of clarithromycin have been shown to cause induction of erm (37). In lower frequency the resistance is associated with mutations in the rrs gene [37]. More recently, it has been shown that mutations in gidB, which encodes a conserved S-adenosylmethionine-dependent 16S rRNA methyltransferase, can confer low-level resistance to streptomycin [38]. Although cross-resistant amikacin and kanamycin, these drugs are not cross-resistant with streptomycin [39, 40].

Ethionamide (ETH) is a pro-drug, structurally related to INH, requiring activation by the monooxygenase EthA. ETH binding ACP reductase InhA inhibiting mycolic acid synthesis. ETH-resistant clinical isolates contains mutations in ethA or inhA [41]. Other potential mutation involved in ETH resistance is mshA deletion, involved in defective activation of the drug [42].

Fluoroquinolones exert their antibacterial activity in M. tuberculosis by trapping gyrase on DNA as ternary complexes, thereby blocking the movement of replication forks and transcription complexes [43]. Fluoroquinolone resistance in M. tuberculosis is most commonly associated with mutations in the conserved quinolone resistance-determining region (QRDR) of gyrA and gyrB involved in the interaction between the drug and DNA gyrase [44]. The degree of fluoroquinolone resistance is dictated by the specific amino acid substitution in the QRDR, as well as the number of resistance mutations present. Therefore, while individual mutations in gyrA may confer low-level resistance (MIC > 2 mg/L) [45], high-level resistance to fluoroquinolones usually requires multiple mutations in gyrA, or concurrent mutations in gyrA and gyrB [46, 47]. The most frequently observed mutations associated with fluoroquinolone resistance in M. tuberculosis are at positions Ala-90 and Asp-94 in the gyrA gene. Interestingly, mutations at position 80 of gyrA have been reported to cause hypersusceptibility to fluoroquinolones, especially when present with other resistance mutations [47]. Since mutations in the QRDR region of gyrA are identified in only 42–85% of fluoroquinolone-resistant clinical isolates.

The macrolides are broad-spectrum antibiotics, which exert their antibacterial effect by binding to the bacterial 50S ribosomal subunit and inhibiting RNA-dependent protein synthesis [48]. Intrinsic resistance to the macrolides in M. tuberculosis has been attributed to low cell wall permeability and expression of the erm(37) gene encoding a 23S rRNA methyltransferase, which is present in all members of the M. tuberculosis complex but absent in nontuberculous mycobacteria [49]. Subinhibitory concentrations of clarithromycin have been shown to cause induction of erm(37) expression and a 4- to 8-fold increase in MIC [50].

3. Leprosy - Drug Resistance Mechanism

The drug resistance in M. leprae can be due to chromosomal mutation in genes encoding drug targets; these mutations occur as a result of errors in DNA replication, and these mutants are enriched in a population of susceptible M. leprae by inappropriate drug therapy. Resistant M. leprae mutants can be acquired during the initial infection from an infection source containing drug-resistant leprosy (primary drug resistance) or from inadequate treatment (secondary drug resistance) [51-55]. The first cases of resistance to dapsone were detected from Malaysia in 1964 [56, 57] and involved two single nucleotide polymorphisms (SNPs) in the gene folP1, located in codons 53 and 55. Dihydropteroate synthase (DHPS) enzyme has been known as a target of Dapsone, an enzyme in the folate biosynthesis pathway in M. leprae. Dapsone inhibits folate acid biosynthesis by acting as a competitive inhibitor of p-aminobenzoic acid (PABA). The development of dapsone resistance is associated to specific mutations within the PABA binding site of E. coli’s DHPS, encoded by folP [51, 58-60]. M. leprae possesses two folP homologues (folP1 and folP2) [61]. Mutations in codon 53 and 55 in folP1 have been associated with high or intermediate levels of dapsone resistance in over 90% of the isolates in different studies [62-66]. However, mutations in folP2 were not found to be associated with dapsone resistance [67]. FolP1 is part of an operon containing three other genes involved in folate biosynthesis, if a mutation occurs in this gene,
the protein arrangement and the enzyme will change. The final result of this is the failure of Dapsone to inhibit the new enzyme, which means that the bacilli become resistant to this drug [56].

Rifamycin is the key bactericidal component of all recommended antileprosy chemotherapeutic regimens. The molecular mechanism of rifampin resistance was first described in *Escherichia coli* [65,68] and was elucidated in 1993 for *M. leprae* [69] and *M. tuberculosis* [70]. The target for rifampin in mycobacteria is the beta (β) subunit of the RNA polymerase encoded by rpoB. Comparison of the deduced primary structures of β-subunit proteins from several bacteria to that of *M. leprae* demonstrated that *M. leprae* shares six highly conserved functional regions common to this enzyme in bacteria [51,68,69]. Mycobacterial, including *M. leprae*, resistance to rifampin correlates with changes in the structure of the β-subunit of the DNA-dependent RNA polymerase primarily due to missense mutations within codons of a highly conserved region of the rpoB gene, referred to as the rifampin resistance determining region (RRDR) [52, 70]. These mutations, that includes codons 407–427, diminish rifampicin-binding affinity for the polymerase [71, 72]. Mutations at codon 407, 410, 420, 425 and insertions between 408 and 409 have been confirmed as associated with rifampicin resistance [63,69, 72-74]. Mutations at codons 401,416 and 427 have also been found, but it has not been revealed clearly whether these mutations confer rifampicin resistance in *M. leprae*. The most commonly found mutation in the RRDR is Ser425Leu [53, 62, 63, 72, 75].

Clofazimine possesses antimycobacterial activities for which the mechanism is not fully elucidated. Clofazimine was first used for leprosy treatment in 1962 and it is highly lipophilic and appears to bind preferentially to mycobacterial. Binding of the drug to DNA appears to occur principally at DNA base sequences containing guanine, which may explain clofazimine preference for the G + C rich genomes of mycobacteria over human DNA. The accumulation of lysophospholipids (detergent-like agents with membrane-disruptive properties in bacterial cells) appears to mediate the activity of clofazimine in some Gram-positive bacteria. However, it is unclear whether this mechanism of action is operational in *M. leprae*. [51, 66, 76]. Since clofazimine may act through several different mechanisms, this may explain the fact that the drug resistance in leprosy is rare [66]. However, no molecular background for drug resistance to clofazimine is known.

Ofloxacin has moderate bactericidal activity for *M. leprae*, first demonstrated in 1968. Its mechanism of action on *M. leprae* is unknown, but in other bacteria it binds to the A subunit of DNA gyrase (gyrA) and inhibits DNA [16]. Mutations within a highly conserved region of gyrA, the quinolone resistance-determining region (QRDR), coded by the gyrA gene are associated with the development of ofloxacin resistance in the majority of resistant strains of *M. tuberculosis*. The first ofloxacin-resistant *M. leprae* was found in 1994 trial [51, 66, 77, 78]. The DRDR of *M. leprae* gyrA is highly homologous to that of *M. tuberculosis*, and missense mutations Ala-Val at codon 91 of this region have been found in the major of ofloxacin-resistant strains of *M. leprae*. However, based on knowledge of *M. tuberculosis* gyrA mutations, mutations at codons 89, 92 and 95 in gyrA of *M. leprae* also confer resistance [63,72,73,78].

Minocycline is bactericidal for *M. leprae* and its activity is additive when it is combined with dapsone and rifampicin. Minocycline inhibits protein synthesis by binding to the 30S ribosomal subunit, blocking the binding of aminoacyl-tRNA to the mRNA ribosome complex [51,79]. The molecular mechanism of minocycline resistance has not been studied in *M. leprae* due to the lack of resistant mutants.

### 4. Leishmaniasis - Drug Resistance Mechanism

Leishmaniasis, caused by protozoan parasites (*Leishmania* genus, order Kinetoplastida), is a major health problem and a neglected disease in many regions of the world. Leishmaniasis occurs on five continents and is considered endemic in 98 countries and three territories, most of which are low- and middle-income [80,81]. The ailment affects an estimated 12 million people worldwide and no vaccine is available. *Leishmania* is responsible for a wide spectrum of diseases, including cutaneous (CL), mucocutaneous (ML) and visceral leishmaniasis (VL). In the absence of an effective vaccine, the control of leishmaniasis is essentially dependent on chemotherapy and vector control [82,83]. However, the chemotherapeutic arsenal is limited and unfortunately, this current small inventory of available drugs to treat leishmaniasis is far from ideal, mainly because of toxicity and therapeutic unresponsiveness [84].

Over 60 years, the pentavalent antimony (SbV) successfully constituted the first-line treatment of leishmaniasis [85]. However, cases refractory to antimony treatment have been described for a long time in humans [1], the treatment failure is attributed to antimony-resistant parasites [86,87]. However, in countries that cannot afford other effective therapies such alkylphospholipid compound miltefosine, liposomal amphotericin B and paromomycin that have higher costs, first-line treatment still depends on sodium stibogluconate (pentosan) or N-methyl glucamine [88]. Regarding, combination therapy may reduce both the treatment duration and the chance on the emergence of drug-resistant parasites while still guaranteeing an excellent efficacy, as shown by the first clinical trial of different combination regimens against VL in the Indian subcontinent [89].

Although the mechanism of action of antimony is still unclear, SbV is a pro-drug which is reduced to the active trivalent form (SbIII) that can act in host macrophage and in the intracellular *Leishmania* amastigote, and it also can activate macrophages [90]. SbIII induces apoptotic like features including accumulation of reactive oxygen species (ROS), drop in mitochondrial potential, genomic DNA degradation and increase in intracellular Ca2+ [90].
Several studies dealing with drug resistance in Leishmania have highlighted the plasticity of the Leishmania genome [91,92]. Mechanisms contributing to drug resistance in vivo are poorly understood. However, molecules, involved in trivalent antimony or arsenite compounds transport like aquaglyceroporins (AQP1) whose down regulation provides resistance to trivalent antimony and mutation could affect specifically metal transport [93, 94]. Some authors reported that terminal deletion of 67 kb to 204 kb in chromosome 31 of Leishmania, hence decreasing the copy number and expression of AQP1 led to SbIII resistance [90]. In addition, over expression of energy dependent transporters seems to play a major role in resistance to antimonials [95]. Genes coding for “ATP binding cassette” (ABC) transporters [96] have been shown to be amplified as extrachromosomal elements in strains selected in vitro for resistance to heavy-metals. For example, LABCI4, a new intracellular ATP-binding cassette (ABC) half-transporter in Leishmania major that is involved in heavy metal export, thereby conferring resistance to Pentostam®, SbIII and to AsIII and CdIII [97]. In addition, Leishmania over-expressing LABCI4 showed a lower mitochondrial toxic effect of antimony by decreasing ROS production, and maintained higher values of both the mitochondrial electrochemical potential and total ATP levels with respect to controls [97]. Additionally, this same studies also was showed that LABCI4 has a significant ability to efflux thiol after SbIII incubation, meaning that LABCI4 could be considered a potential thiol-X-pump that is able to recognize metal-conjugated thiols [97].

The antimony resistance also is associated to the amplification of genes involved in pathways of detoxification of SbIII via conjugation to the unique parasitic di-thiol trypanothione (T[SH]2), and subsequent sequestration of the metal–thiol conjugate into vesicular membranes of Leishmania by a specific ABC protein transporter (MRPA) [98,99]. SbV resistance L. donovani strains has a higher gene expression of the host cell’s multidrug resistance-associated protein 1 (MRP1) and permeability glycoprotein (P-gp), which can export SSG out of the host cell [90].

Miltefosine (MIL), an alkylphosphocholine originally developed as an anticancer drug, has been used since 2005 in first line for the oral treatment of VL in the Indian subcontinent, currently is an alternative to antimonials resistance Leishmania strains [100, 101]. MIL resistance is easily induced by growing Leishmania parasites under increasing drug concentrations [102]. The resistance mechanism is associated to overexpressed efflux pumps and failure of the MIL-dedicated transporter, the aminophospholipid translocase LdMT (Leishmania donovani miltefosine transporter). In this regards, substantial low-Mutations in LdMT, an inwards translocator of MIL, or its beta subunit LdRos3 were found to be responsible for in vitro-induced MIL resistance [103]. In addition, a new single-nucleotide polymorphism (SNP), L832F, was identified, which might be a marker of miltefosine resistance in leishmaniasis [104].

5. Fungal Infections - Drug Resistance Mechanism

The fungal kingdom includes a diverse number of taxa with varied ecological niches, life-cycle strategies, as well as morphological aspects [105]. Many of these fungi can parasitise different hosts, such as plants, animals or even humans. In human, some fungi can cause serious diseases, of which may be fatal if left untreated. During the last decades, drug resistance has become an important problem in a variety of infectious diseases including human immunodeficiency virus (HIV) infection, tuberculosis, and fungal infections which has producing profound effects on human health[106]. Several factors have contributed to the increase of life-threatening invasive fungal infections as an important cause of morbidity and mortality in immunosuppressed individuals and those undergoing invasive procedures [107,108].

The incidence of invasive fungal infections are particularly caused by Candida species, Cryptococcus neoforms, and other less common fungi, both yeasts and filamentous fungi, many of which are intrinsically resistant to the actually available antifungal drugs, and is increasingly recognized as opportunistic pathogens, such as Aspergillus spp, Trichosporon spp, Fusarium spp, and others. In this context, yeasts are the most common opportunistic pathogenic fungus in human and important organisms of nosocomial infection. They are frequently found in AIDS patients, bone marrow transplant patients and patient cancer chemotherapy.

The increase survival of critically ill patients have lead at development of the new antifungal drugs against new molecular targets to combat the rising of infection and emergence resistance, since many of the currently available drugs have undesirable side effects, or are not effective against emerging fungi pathogen, and contribute to the rapid development of resistance. In general, fungi can be intrinsically resistant to antifungal drugs (primary resistance) or can develop resistance in response to exposure to the drug during treatment (secondary resistance) [109].

Current choice drugs include polyenes, of which amphotericin B is the most commonly used for treating invasive fungal infection disease, as well nucleosome analogue and azoles. In this case, has been observed an increase in the use of azoles prophylactically for high-risk individuals due to concerns of developing fungal infections or to treat patients who have already acquired fungal disease. The large scale use of azole compound and fungistatic nature has led to the emergence of resistance in clinical isolates [110,111].

The most majority of the antifungal drugs in clinical use target ergosterol in the fungal cell membrane, the biosynthesis of ergosterol, or the biosynthesis of (1,3)-beta-D-glucan, a major component of the fungal cell wall. Ergosterol is an important constituent of membrane lipids, similar to vertebrate cholesterol, and modulates the cell morphology, the membrane fluidity and permeability. These sterols preferentially associate with sphingolipids in microdomains that have been postulated to have important roles in membrane organization and function. This compound act by interfering with the structural or functional integrity of the fungal plasma membrane [112,113,114].
Currently, there are four triazole drugs available for clinical use, fluconazole, itraconazole, voriconazole, and posaconazole, each with its own pharmacokinetic properties [114]. The azoles generally act in a fungistatic manner against yeasts, including Candida species. The fungistatic nature of azoles toward Candida imposes strong directional selection on surviving populations to evolve drug resistance [115,116]. The azoles affect the biosynthesis of ergosterol, the major sterol in the fungal plasma membrane, by inhibiting 14-α- lanosterol demethylase, the product of the ERG11 gene. In many fungal species, they enter the fungal cell by facilitated diffusion[117], and act through an unhindered nitrogen atom in theazole ring, which binds to an iron atom in the heme group located in the active site of Erg11[113]. This inhibits the activation of oxygen, which is necessary for the demethylation of lanosterol, blocking the production of ergosterol and resulting in the accumulation of 14-α-methyl-3,6-diol, a toxic compound intermediate produced by the Δ5,6-desaturase encoded by ERG3[118]. This toxic sterol exerts severe membrane stress on the cell. Recently, it was also shown that the azoles impair the function of vacuolar membrane H+ATPases, thereby disrupting cation homeostasis within the cell and providing a mechanistic insight into the cellular consequences of ergosterol depletion[112].

In C. albicans, several mechanisms of resistance have been well characterized [113]. In relation to the azoles, C. albicans can acquire resistance through multiple mechanisms, such as up regulation the ERG11 gene, which encodes the azole target lanosterol demethylase [119]; the up regulation of the multidrug transporter Cdr1, Cdr2, or Mdr1 (fluconazole specific) is the most-frequently encountered triazole resistance mechanism in clinical isolates [120]; or the induction of numerous cellular stress responses (calcineurin-mediated) [115]. Regarding the echinocandin by inhibiting the synthesis of (1,3)-β-D-glucan, cause loss of cell wall integrity and induce an acute cell wall stress, leading at compensatory synthesis of cell wall components, by means protein kinase C (PKC) cell wall integrity signaling pathway that is responsible for remodeling the cell wall periodically through the cell cycle and in response to various stresses [115,121].

In summary, the resistance phenomenon observed in some fungi, mainly in C. albicans, may occur by increased levels of the cellular target, up regulation of genes controlling drug efflux, or alterations in sterol synthesis and decreased affinity of azoles for the cellular target. And it is well established that antifungal resistance in fungi is not restricted to a single mechanism but is rather a multifactorial phenomenon.

6. Cryptococcosis - Drug Resistance Mechanism

Cryptococcus neoformans is an important human fungal pathogen involved in the etiology of meningitis and pulmonary disease, as also as, in disseminated infections in immunocompromised patients. In this particular group, C. neoformans has a major role in morbidity and mortality rates worldwide [122]. In HIV-infected patients, it is estimated that 1 million cases of cryptococcal meningitis is causes by C. neoformans annually, especially in sub-Saharan Africa [123]. C. neoformans has been shown to present different susceptibility profiles to antifungal drugs according to in vitro studies, although little reports of resistant cases have been described [124]. C. neoformans drug resistance can be seen to azoles, especially fluconazole, to amphotericin B (AmB) and to 5-flucytosine (5FC).

Among the azoles, fluconazole is one of the most used antifungal for the cryptococcal meningitis treatment due to its large availability in developing countries, particularly those in Asia and Africa, in which cryptococcal meningitis is more prevalent [125]. Fluconazol, inhibits the ergosterol biosynthesis pathway by targeting specifically the cytochrome P-450 lanosterol 14-alpha-demethylase, encoded by the ERG gene family [126]. Point mutation in the ERG11 gene leads to complete blockage of the bind ability of fluconazole to its target [127]. Long treatment of cryptococcal infections by azoles can lead to resistance and one of the molecular mechanisms involves SRE1 gene which was shown to regulate the ergosterol biosynthesis pathway and to be necessary for C. neoformans growth in the presence of low level of azoles [128]. Other six genes (SFB2, STP1, SCP1, KAP123, GSK3, and DAM1) were also identified in C. neoformans as responsible to play a role in the sterol regulatory element-binding protein pathway that is necessary for host adaptation and virulence [129].

Amphotericin B (AmB) is a polyene macrolide antibiotic that binds to ergosterol disrupting the osmotic balance of the fungal membrane [130]. C. neoformans resistance to AmB is a very rare event and is estimated to be around 1% in clinical isolates [124]. Acquired or innate resistance to AmB is frequently related with modification of membrane lipids, particularly ergosterol, by a decrease of its amount in plasmalemma or an alteration in the target lipid, which reduces the binding of the drug [131]. This reduction is caused by mutations in nonessential genes of the ergosterol biosynthesis pathway [132]. Besides that, other mechanisms have been found, including a defect in 8–7 isomerase or the azole target lanosterol de methylase [119]; the up regulation of the multidrug transporter Cdr1, Cdr2, or Mdr1, (fluconazole specific) is the most-frequently encountered triazole resistance mechanism in clinical isolates [120]; or the up regulation of nonessential genes of the ergosterol biosynthesis pathway [133].

5-Flucytosine (5-FC) belongs to the class of pyrimidine analogues and C. neoformans resistance is mostly seen during monotherapy and due to this fact it’s commonly used in combination with another antifungal, such as AmB [134]. Resistance to 5-FC can be regulated by MBS1 gene. Studies showed that C. neoformans with the deletion of MBS1 exhibits increased susceptibility to 5-FC [135]. Other frequently resistance mechanism found to 5-FC is a point mutation in the FUR1 gene that encodes the enzyme responsible for the conversion of the drug into metabolites able to enter the cytosine metabolism, leading to a complete resistance to 5-FC [136].
7. Conclusion

Neglected diseases are mostly endemic in underdeveloped and developing countries, and a global health problem with high incidence due to poor preventative health care and lack of effective vaccines. In addition, the parasite genome plasticity offers several solutions to minimize the stress induced by drugs, leading to activation of multiple mechanisms of resistance to the various therapies. Thus, molecular studies on the mechanism of action of drugs have elucidated the genetic basis of drug resistance of parasites and have support to developing of molecular tests to assess drug resistance. Currently, the application of molecular techniques for detection and characterization of mutations has been widely used and present viable for the diagnosis of resistance. However, this is still not a reality for some laboratories that have limited resources, which often have no access to even simple techniques such as PCR. Therefore, if there implementation of a rapid detection of drug resistance by molecular methods would be permit treatment early of patients, and thus to avoid dissemination of resistant strains.

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