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 other. 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

pH [20]. The PZA resistance-associated mutations have been found in the putative promoter region or the structural gene pncA that encodes PZase [21, 22]. About 3% of all PZA-resistant *M. tuberculosis* no have mutations in this gene, suggesting alternative mechanisms of EMB resistance.

The primary inhibitory action of ethambutol (EMB) appears to be the inhibition of cell wall arabinan polymerization, although the EMB have been reported to inhibit several other cellular pathways, including RNA metabolism, transfer of mycolic acids into the cell wall, phospholipid synthesis and spermidine biosynthesis [23, 24, 25, 26, 27]. Resistance to EMB in *M. tuberculosis* is usually associated with point mutations in the embCAB operon [28]. Genetic and biochemical studies have shown that the EmbA and EmbB proteins are involved in the formation of the proper terminal hexaarabinofuranoside motif during arabinogalactan synthesis [29], while EmbC is involved in lipoarabinomannan synthesis [30]. The mutation in codon 306 have been reported to be associated with variable degrees of EMB resistance, maybe that such mutations may be necessary but not sufficient for high- level EMB resistance. Other potential mutations involved in EMB resistance include codon 379 embR, and mutations in the rmlD, rmlA2, and Rv0340 genes. About 25% of all EMB-resistant *M. tuberculosis* isolates no have mutations in any of the genes described above [31, 32, 33, 34]

The mechanism of action of all aminoglycosides is binding to the 30S ribosomal subunit, affecting polypeptide synthesis. The resistance to streptomycin and the other aminoglycosides in *M. tuberculosis* is usually associated by mutation of the gene that encode ribosome target binding sites or in rpsL gene, which encodes the ribosomal protein S12, [35, 36]. 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- resistance is observed between 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 syntesis. 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 sin 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 the *M. leprae*.

Dapsone inhibits folic acid biosynthesis by acting as a competitive inhibitor of p-aminobenzoic acid (PABA). The development of dapsone resistence 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[70, 71, 52]. 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 most 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 majority 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 CdII [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 the amplification of genes involved in pathways of detoxification of SbIII via conjugation to the unique parasitic dithiol 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 neoformans*, 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 nucleoside 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 the azole 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-alfa-methyl-3,6-diol, a toxic compound intermediate produced by the Δ-5,6-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)-beta-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 criptococcal 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].

Amphotecin 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 in a clinical *C. neoformans* isolate from an AIDS patient [133].

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.

References

[1] Croft, S. L., Sundar, S. and Fairlamb, A. H. (2006). Drug resistance in leishmaniasis. Clinical Microbiology Reviews 19, 111–126.

[2] Kwon HH, Tomioka H, Saito H: Distribution and characterization of beta-lactamases of mycobacteria and related organisms. Tuber Lung Disease 1995, 76:141–148.

[3] Vester B, Douthwaite S: Macrolide resistance conferred by base substitutions in 23S rRNA. Antimicrobial Agents Chemotherapy. 2001, 45:1–12.

[4] Zhang Y, Telenti A: Genetics of drug resistance in Mycobacterium tuberculosis. In: Molecular Genetics of Mycobacteria. Edited by Hatful GF, Jacobs WR Jr. Washington DC: ASM Press; 2000:235–254.

[5] Heym B, Honore N, Truffot-Pernot C, Banerjee A, Schurra C, Jacobs WR Jr, van Embden JD, Grosset JH, Cole ST: Implications of multidrug resistance for the future of short-course chemotherapy of tuberculosis: a molecular study. Lancet 1994, 344:293–98.

[6] Suter E. Multiplication of tubercle bacilli within phagocytes cultivated in vitro, and effect of streptomycin and isonicotinic acid hydrazide. Am. Rev. Tuberc. 1952. 65, 775-776.

[7] Mackaness GB, Smith N. The action of isoniazid (isonicotinic acid hydrazide) on intracellular tubercle bacilli. Am. Rev. Tuberc., 195266, 125-133.

[8] Bardou F, Raynaud C, Ramos C, Lanéelle MA, Lanrelle G. Mechanism of isoniazid uptake in Mycobacterium tuberculosis. Microbiology. 1998. 144 (Pt 9): 2539-2544

[9] Winder, FG, Collins PB. Inhibition by isoniazid of synthesis of mycolic acids in Mycobacterium tuberculosis. Journal of General Microbiology. 1970. 63(1): 41-48.

[10] Takayama K, Schnoes H K, Armstrong EL, Boyle RW. Site of inhibitory action of isoniazid in the synthesis of mycolic acids in Mycobacterium tuberculosis. Journal of Lipid Research. 1975.16(4): 308- 317.

[11] Gangadharam PR, Harold FM, Schaefer WB. Selective inhibition of nucleic acid synthesis in Mycobacterium tuberculosis by isoniazid." Nature.1963. 198: 712-714.

[12] Zatman LJ, Kaplan NO, Colowick SP, and Ciotti MM. Effect of isonicotinic acid hydrazide on diphosphopyridine nucleotidases. J Biol Chem 1954. 209: 453-466.

[13] Banerjee A, Dubnau E, Quemard A, Balasubramanian V, Um KS, Wilson T, Collins D, Lisle G, Jacobs Jr WR. inhA, a gene encoding a target for isoniazid and ethionamide in Mycobacterium tuberculosis. Science 1994. 263(5144): 227-230.

[14] Quemard, A., J. C. Sacchettini, Dessen A, Vilchèzes C, Bittman R, Jacobs WR, Jr., Blanchard JS. Enzymatic characterization of the target for isoniazid in Mycobacterium tuberculosis. Biochemistry. 1995. 34(26): 8235-8241.

[15] Mani C, Selvakumar N, Narayanan S, Narayanan PR.Mutations in the rpoB Gene of Multidrug-Resistant Mycobacterium tuberculosis Clinical Isolates from India J Clin Microbiol. 2001 August; 39(8): 2987–2990.

[16] Konno K, Feldmann F M, McDermott W. Pyrazinamide susceptibility and amidase activity of tubercle bacilli. Am Rev Respir Dis. 1967;95:461–469.

[17] Scorpio A, Zhang Y. Mutations in pncA, a gene encoding pyrazinamidase/nicotinamidase, cause resistance to the antituberculous drug pyrazinamide in tubercle bacillus. Nat Med. 1996; 2:662–667.

[18] Mitchison DA. The action of antituberculosis drugs in short-course chemotherapy. Tubercle. 1985. 66(3): 219-225

[19] Raynaud C, Laneelle M A, et al. Mechanisms of pyrazinamide resistance in mycobacteria: importance of lack of uptake in addition to lack of pyrazinamidase activity. Microbiology 1999. 145 (Pt 6): 1359-1367.

[20] Zhang Y, Wade MM, et al. Mode of action of pyrazinamide: disruption of Mycobacterium tuberculosis membrane transport and energetics by pyrazinoic acid. J Antimicrob Chemother. 2003. 52(5): 790-795.

[21] Parsons LM, Driscoll JR, Taber HW, Salfinger M: Drug resistance in tuberculosis. Infect Dis Clin North Am 1997, 11:905–928.

[22] Somoskovi A, Parsons LM, Salfinger M. The molecular basis of resistance to isoniazid, rifampin, and pyrazinamide in Mycobacterium tuberculosis. Respir. Res. 2001. 2:164–168.

[23] Forbes M, Kuck NA, et al. Mode of action of ethambutol. Journal of Bacteriology. 1962. 84: 1099-1103.

[24] Forbes M, Kuck NA, et al. Effect of Ethambutol on Nucleic Acid Metabolism in Mycobacterium smegmatis and Its Reversal by Polyamines and Divalent Cations. Journal of Bacteriology. 1965. 89: 1299-1305.

[25] Paulin LG, Brander EE, et al. Specific inhibition of spermidine synthesis in Mycobacteria spp. by the dextro isomer of ethambutol. Antimicrob Agents Chemother. 1985. 28(1): 157-159.

[26] Takayama K, Armstrong EL, et al. Inhibition by ethambutol of mycolic acid transfer into the cell wall of Mycobacterium smegmatis. Antimicrob Agents Chemother 1979.16(2): 240-242.

[27] Cheema S, Khuller GK.Metabolism of phospholipids in Mycobacterium smegmatis ATCC 607 in the presence of ethambutol. Indian J Med Res. 1985. 82: 207-213.
[28] Belanger, AE, Besra, GS, et al. The embAB genes of Mycobacterium avium encode an arabinosyl transferase involved in cell wall arabinan biosynthesis that is the target for the antimycobacterial drug ethambutol." Proc Natl Acad Sci U S A 1996. 93(21): 11919-11924.
[29] Escuyer VE, Lety MA, et al. The role of the embA and embB gene products in the biosynthesis of the terminal hexaarabinofuranosyl motif of Mycobacterium smegmatis arabinogalactan.J Biol Chem 2001. 276(52): 48854-48862.
[30] Zhang N, Torrelles JB, et al. The Emb proteins of mycobacteria direct arabinosylation of lipoarabinomannan and arabinogalactan via an N-terminal recognition region and a C-terminal synthetic region.Mol Microbiol 2003. 50(1): 69-76.
[31] Karakousis PC. Mechanisms of Action and Resistance of Antimycobacterial Agents. In: Antimicrobial Drug Resistance. 2009. D. L. Mayers, Springer: pp.271-291.
[32] Sreevatsan S, Stockbauer KE, et al. Ethambutol resistance in Mycobacterium tuberculosis: critical role of embB mutations. Antimicrob Agents Chemother .1997.41(8): 1677-1681.
[33] Telenti A, Philipp WJ, et al. The emb operon, a gene cluster of Mycobacterium tuberculosis involved in resistance to ethambutol. Nat Med. 1997. 3(5): 567-570.
[34] Ramaswamy SV, Amin AG, et al. Molecular genetic analysis of nucleotide polymorphisms associated with ethambutol resistance in human isolates of Mycobacterium tuberculosis." Antimicrob Agents Chemother. 2000. 44(2): 326-336.
[35] Nair J, Rouse DA, et al. The rpsL gene and streptomycin resistance in single and multiple drug-resistant strains of Mycobacterium tuberculosis. Mol Microbiol.1993. 10(3): 521-527.
[36] Cooksey RC, Morlock GP, et al. Characterization of streptomycin resistance mechanisms among Mycobacterium tuberculosis isolates from patients in New York City. Antimicrob Agents Chemother. 1996. 40(5): 1186-1188.
[37] Douglass J, Steyn LM. A ribosomal gene mutation in streptomycin-resistant Mycobacterium tuberculosis isolates. Journal of Infectious Diseases. 1993. 167(6): 1505-1506.
[38] Wong, SY, Lee JS, et al. Mutations in gidB confer low-level streptomycin resistance in Mycobacterium tuberculosis. Antimicrob Agents Chemother. 2011. 55(6): 2515-2522.
[39] Tsukamura M, Mizuno S. Cross-resistant relationships among the aminoglucoside antibiotics in Mycobacterium tuberculosis. J Gen Microbiol 1975. 88(2): 269-274.
[40] Allen, B. W., D. A. Mitchison, et al. Amikacin in the treatment of pulmonary tuberculosis. Tubercle. 1983. 64(2): 111-118.
[41] Morlock GP, Metchock B, et al. ethA, inhA, and katG loci of ethionamide resistant clinical Mycobacterium tuberculosis isolates." Antimicrob Agents Chemother. 2003. 47(12): 3799-3805.
[42] Vilcheze C, Av-Gay Y, et al. Mycothiol biosynthesis is essential for ethionamide susceptibility in Mycobacterium tuberculosis." Mol Microbiol. 2008. 69(5): 1316-1329.
[43] Drlica K. Malik M. Fluoroquinolones: action and resistance. Curr Top Med Chem. 2003. 3(3): 249-282.
[44] Ginsburg AS, Grosset, JH et al. Fluoroquinolones, tuberculosis, and resistance. Lancet Infectious Diseases. 2003. 3(7): 432-442.
[45] Kocagoz T, Hackbarth CJ, et al. Gyrase mutations in laboratory-selected, fluoroquinolone-resistant mutants of Mycobacterium tuberculosis H37Ra. Antimicrob Agents Chemother. 1996. 40(8): 1768-1774.
[46] Xu C, Kreiswirth BN. Fluoroquinolone resistance associated with specific gyrase mutations in clinical isolates of multidrug-resistant Mycobacterium tuberculosis. Journal of Infectious Diseases. 1996. 174(5): 1127-1130.
[47] Aubry A, Veziris N, et al. Novel gyrase mutations in quinolone-resistant and -hypersusceptible clinical isolates of Mycobacterium tuberculosis: functional analysis of mutant enzymes." Antimicrob Agents Chemother 2006. 50(1): 104-112.
[48] Piscitelli SC, Danziger LH, et al. Clarithromycin and azithromycin: new macrolide antibiotics. Clin Pharm. 1992. 11(2): 137-152.
[49] Buriankova K, Doucet-Populaire F, et al. Molecular basis of intrinsic macrolide resistance in the Mycobacterium tuberculosis complex." Antimicrob Agents Chemother . 2004. 48(1): 143-150.
[50] Andini N, Nash KA. Intrinsic macrolide resistance of the Mycobacterium tuberculosis complex is inducible. Antimicrob Agents Chemother. 2006. 50(7): 2560-2562.
[51] Williams DL, Gillis TP. Drug-resistant leprosy: monitoring and current status. Lepr Rev. 2012; 83(3):269-81.
[52] Musser JM. Antimicrobial agent resistance in mycobacteria: molecular genetic insights. Clin Microbiol Rev. 1995; 8: 496–514.
[53] Cambau E, Bonnafous P, Perani E et al. Molecular detection of rifampin and ofloxacin resistance for patients who experience relapse of multibacillary leprosy. Clin Infect Di. 2002; 34: 39–45.
[54] Musser J. Antimicrobial agent resistance in mycobacteria: molecular genetic insights. Clin Microbiol. Rev 1995; 8:496–514.
[55] Williams D, Spring L, Harris E, Riche P, Gillis T. Dihydropteroate synthase of Mycobacterium leprae and dapsone resistance. Antimicrob Agents Chemother. 2000; 44:1530–7.
[56] Adriaty D, Wahyuni R, Iswahyudi, Prakoeswa CRS, Abdullah R, Agusni I, Izumi S. Dapsone resistance in a Mycobacterium leprae isolate with two point mutations in folp gene from a leprosy patient.Indonesian Journal of Tropical and Infectious Disease. 2013, 3(2): 108-111.
[57] Pettit JHS and Rees JW (1964). Sulphone resistance in leprosy: an experimental and clinical study. Lancet .1964; 2: 673–4.
[58] Seydel JK, Richter M, Wempe E. Mechanism of action of the folate blocker diaminodiphenylsulfone (dapsone, DDS) studied in E. coli cell-free enzyme extracts in comparison to sulfonamides (SA). Int J Lepr Other Mycobact. Dis. 1980; 48: 18–29.
[59] Richey DP, Brown GM. The biosynthesis of folic acid. IX. Purification and properties of the enzymes required for the formation of dihydropteroic acid. J Biol Chem. 1969; 244: 1582–1592.
[60] Browne SG, Hogerzeil LM. B663 in the treatment of leprosy. Preliminary report of a pilot trial. Lepr Ver. 1962; 33: 6–10.
[61] Cole ST, Eiglmeier K, Parkhill J, James KD, Thomson NR, Wheeler PR, Honoré N, Garnier T, Churcher C, Harris D, Mungall K, Basham D, Brown D, Chillingworth T, Connor R, Davies RM, Devlin K, Duthoy S, Feltwell T, Fraser A, Hamlin N, Holroyd S, Hornsby T, Jagels K, Lacroix C, Maclean J, Moule S, Murphy L, Oliver K, Quail MA, Rajandream MA, Rutherford

KM, Rutter S, Seeger K, Simon S, Simmonds M, Skelton J, Squares R, Squares S, Stevens K, Taylor K, Whitehead S, Woodward J R, Barrell BG. Massive gene decay in the leprosy bacillus. Nature, 2001; 409: 1007–1011.
[62] Singh P, Cole ST. Mycobacterium leprae: genes, pseudogenes and genetic diversity. Future Microbiol. 2011, 6(1):57-71.
[63] Matsuoka M, Budiawan T, Aye KS, Kyaw K, Tan EV, Cruz ED, Gelber R, Saunderson P, Balagon V, Pannikar V. The frequency of drug resistance mutations in Mycobacterium leprae isolates in untreated and relapsed leprosy patients from Myanmar, Indonesia and the Philippines. Lepr. Rev. 2007, 78(4):343–352.
[64] Lee SB, Kim SK, Kang TJ, Chae GT, Chun JH, Shin HK, Kim JP, Ko YH, Kim NH.The prevalence of folP1 mutations associated with clinical resistance to dapsone, in Mycobacterium leprae isolates from South Korea. Ann. Trop. Med. Parasitol. 2001, 95(4):429–432.
[65] Cambau E, Carthagena L, Chauffour A, Ji B, Jarlier V. Dihydropteroate synthase mutations in thefolP1 gene predict dapsone resistance in relapsed cases of leprosy. Clin. Infect. Dis. 2006, 42(2): 238–241.
[66] Matsuoka M. Drug resistance in leprosy. Jpn J Infect Dis. 2010, 63(1):1-7.
[67] Gillis TP, William DL. Dapsone resistance does not appear to be associated with a mutation in the dihydropteroate synthase-2 of Mycobacterium leprae. Indian J. Lepr. 1999, 71:11–18.
[68] Jin D, Gross C. Mapping and sequencing of mutations in the Escherichia coli rpoB gene that lead to rifampin resistance. J Mol Biol. 1988, 202: 45–58.
[69] Honore´ N, Cole ST. Molecular basis of rifampin resistance in Mycobacterium leprae. Antimicrob Agents Chemother. 1993, 37:414–8.
[70] Telenti A, Imboden P, Marchesi F, Schmidheini T, Bodmer T. Direct, automated detection of rifampin-resistant Mycobacterium tuberculosis by polymerase chain reaction and single-strand conformation polymorphism analysis. Antimicrob Agents Chemother. 1993, 37:2054–8.
[71] Kumar MM, Shrawan K, Ram D, Kumar GR. Rapid detection of rifampicin resistance in sputum samples using indigenously developed molecular probes and comparison with conventional mic method. Journal of Advance Researches in Biological Sciences. 2013, 5(1: 19-22.
[72] Maeda S, Matsuoka M, Nakata N et al. Multidrug resistant Mycobacterium leprae from patients with leprosy. Antimicrob Agents Chemother. 2001, 45: 3635–3639.
[73] Matsuoka M, Kashiwabara Y, Namisato M. A Mycobacterium leprae isolate resistant to dapsone, rifampin,ofloxacin and sparfloxacin. Int J Lepr. 2000 68: 452–455.
[74] Williams DL, Waguespack C, Eisenack K et al. Chracterization of reifampin resistance in pathogenic Mycobacteria. Antimicrob Agents Chemother. 1994; 38: 2380–2386.
[75] Honore N, Roche P, Grosset J, Cole ST. A method for rapid detction of rifampicin resitant isolates of Mycobacterium leprae. Lepr Rev. 2001, 72: 441–448.
[76] Browne SG, Hogerzeil LM. " B 663" in the treatment of leprosy. Preliminary report of a pilot trial. Leprosy review. 1962, 33: 6-10.
[77] Takiff HE, Salazar L, Guerrero C et al. Cloning and nucleotide sequence of Mycobacterium tuberculosis gyrA and gyrB genes and detection of quinolone resistance mutations. Antimicrob Agents Chemother. 1994, 38: 773–780.
[78] Cambau E, Perani E, Guillemin I, Jamet P, Ji B. Multidrug-resistance to dapsone, rifampicin, and ofloxacin in Mycobacterium leprae. The Lancet. 1997, 349 (9045): 103-104.
[79] Taylor DE, Chau A. Tetracycline resistance mediated by ribosomal protection. Antimicrob Agents Chemother. 1996, 40: 1–5.
[80] WHO - World Health Organization, ―Control of the leishmaniases‖, World Health Organization technical report series , vol. 949, pp. 1-186, 2010.
[81] J. Alvar, I. D. Vélez, C. Bern, M. Herrero et al., ―Leishmaniasis worldwide and global estimates of its incidence‖, PLoS ONE 7: e35671. doi:10.1371/journal.pone.0035671, 2012.
[82] Singh, B. and Sundar, S. (2012). Leishmaniasis: vaccine candidates and perspectives. Vaccine 30, 3834–3842.
[83] Singh, N., Kumar, M. and Sing, R. K. (2012). Leishmaniasis: current status of available drugs and new potential drug targets. Asian Pacific Journal of Tropical Medicine 5, 485–497.
[84] Roberts SC. The genetic toolbox for Leishmania parasites. Bioeng Bugs. 2011 Nov-Dec;2(6):320-6.
[85] Berg M, Mannaert A, Vanaerschot M, VAN DER Auwera G, Dujardin JC. (Post-) Genomic approaches to tackle drug resistance in Leishmania. Parasitology. 2013 Mar 12:1-14.
[86] Lira, R., Sundar, S., Makharia, A., Kenney, R., Gam, A., Saraiva, E., and Sacks, D. (1999) Evidence that the high incidence of treatment failures in Indian kala-azar is due to the emergence of antimony-resistant strains of Leishmania donovani. J Infect Dis 180: 564–567.
[87] Sundar, S. (2001) Drug resistance in Indian visceral leishmaniasis. Trop Med Int Health 6: 849–854.
[88] Jha TK, Sundar S, Thakur CP, Bachmann P, Karbwang J, Fischer C, Voss A, Berman J. Miltefosine, an oral agent, for the treatment of Indian visceral leishmaniasis. N Engl J Med. 1999 Dec 9;341(24):1795-800.
[89] Sundar S, Sinha PK, Rai M, Verma DK, Nawin K, Alam S, Chakravarty J, Vaillant M, Verma N, Pandey K, Kumari P, Lal CS, Arora R, Sharma B, Ellis S, Strub-Wourgaft N, Balasegaram M, Olliaro P, Das P, Modabber F. Comparison of short-course multidrug treatment with standard therapy for visceral leishmaniasis in India: an open-label, non-inferiority, randomised controlled trial. Lancet. 2011 Feb 5;377(9764):477-86.
[90] Mukherjee A, Boisvert S, Monte-Neto RL, Coelho AC, Raymond F, Mukhopadhyay R, Corbeil J, Ouellette M. Telomeric gene deletion and intrachromosomal amplification in antimony-resistant Leishmania. Mol Microbiol. 2013 Apr;88(1):189-202.
[91] A. Bryceson, "A policy for leishmaniasis with respect to the prevention and control of drug resistance," Tropical Medicine and International Health, 2001; 6(11): 928–934.
[92] M. Gramiccia, L. Gradoni, and S. Orsini, "Decreased sensitivity to meglumine antimoniate (Glucantime) of Leishmania infantum isolated from dogs after several courses of drug treatment," Annals of Tropical Medicine and Parasitology, 1992; 86 (6): 613–620.

[93] N. L. Uzcategui, Y. Zhou, K. Figarella, J. Ye, R.Mukhopadhyay, and H. Bhattacharjee, Alteration in glycerol and metalloid permeability by a single mutation in the extracellular C-loop of Leishmania major aquaglyceroporin LmAQP1," Molecular Microbiology, vol. 70, no. 6, pp. 1477–1486, 2008.
[94] Mary C, Faraut F, Deniau M, Dereure J, Aoun K, Ranque S, Piarroux R. Frequency of drug resistance gene amplification in clinical leishmania strains. Int J Microbiol. 2010;2010. pii: 819060.
[95] J. M. P´erez-Victoria, A. Parodi-Talice, A. Torres, F. Gamarro, and S. Castanys, "ABC transporters in the protozoan parasite Leishmania," International Microbiology, vol. 4, no. 3, pp. 159–166, 2001.
[96] A. Haimeur, C. Brochu, P.-A. Genest, B. Papadopoulou, and M. Ouellette, "Amplification of the ABC transporter gene PGPA and increased trypanothione levels in potassium antimonyl tartrate (SbIII) resistant Leishmania tarentolae, Molecular and Biochemical Parasitology, vol. 108, no. 1, pp. 131–135, 2000.
[97] Manzano JI, García-Hernández R, Castanys S, Gamarro F. A new ABC half-transporter in Leishmania is involved in resistance to antimony. Antimicrob Agents Chemother. 2013 May 28. [Epub ahead of print]
[98] Grondin K, Haimeur A, Mukhopadhyay R, Rosen BP, Ouellette M. Co-amplification of the gamma-glutamylcysteine synthetase gene gsh1 and of the ABC transporter gene pgpA in arsenite-resistant Leishmania tarentolae. EMBO J. 1997 Jun 2;16(11):3057-65.
[99] Haimeur A, Brochu C, Genest P, Papadopoulou B, Ouellette M. Amplification of the ABC transporter gene PGPA and increased trypanothione levels in potassium antimonyl tartrate (SbIII) resistant Leishmania tarentolae. Mol Biochem Parasitol. 2000 Apr 30;108(1):131-5.
[100] Luque-Ortega JR, de la Torre BG, Hornillos V, Bart JM, Rueda C, Navarro M, Amat-Guerri F, Acuña AU, Andreu D, Rivas L. Defeating Leishmania resistance to miltefosine (hexadecylphosphocholine) by peptide-mediated drug smuggling: a proof of mechanism for trypanosomatid chemotherapy. J Control Release. 2012; 10;161(3):835-42.
[101] Sundar S, Rai M, Chakravarty J, Agarwal D, Agrawal N, Vaillant M, Olliaro P, Murray HW. New treatment approach in Indian visceral leishmaniasis: single-dose liposomal amphotericin B followed by short-course oral miltefosine. Clin Infect Dis. 2008 Oct 15;47(8):1000-6.
[102] F.J. Pérez-Victoria, S. Castanys, F. Gamarro, Leishmania donovani resistance to miltefosine involves a defective inward translocation of the drug, Antimicrob. Agents Chemother. 47 (2003) 2397–2403.
[103] Pérez-Victoria JM, Cortés-Selva F, Parodi-Talice A, Bavchvarov BI, Pérez-Victoria FJ, Muñoz-Martínez F, Maitrejean M, Costi MP, Barron D, Di Pietro A, Castanys S, Gamarro F. Combination of suboptimal doses of inhibitors targeting different domains of LtrMDR1 efficiently overcomes resistance of Leishmania spp. to Miltefosine by inhibiting drug efflux. Antimicrob Agents Chemother. 2006 Sep;50(9):3102-10.
[104] Cojean S, Houzé S, Haouchine D, Huteau F, Lariven S, Hubert V, Michard F, Bories C, Pratlong F, Le Bras J, Loiseau PM, Matheron S. Leishmania Resistance to Miltefosine Associated with Genetic Marker. Emerg Infect Dis. 2012 Apr;18(4):704-6.
Candida
[105] Vandeputte P, Ferrari S, Coste AT. Antifungal resistance and new strategies to control fungal infections. International Journal of Microbiology. 2012, Article ID 713687, 26 pages doi:10.1155/2012/713687.
[106] White TC, Marr KA, Bowden RA. Clinical, Cellular, and Molecular Factors That Contribute to Antifungal Drug Resistance. Clinical Microbiology Review. 1998, 11(2):38-402.
[107] Bodey GP. The emergence of fungi as major hospital pathogens. The Journal Hospital Infection. 1988; 11 (Suppl. A): 411-26.
[108] Anaissie E. Opportunistic mycoses in the immunocompromised host: Experience at a cancer center and review. Clinical Infectious Disease. 1992; 14 (Suppl. 1): s43-53.
[109] Perea S, Patterson TF. Antifungal Resistance in Pathogenic Fungi. Clinical Infectious Diseas,. 2002. 35:1073–1080.
[110] Sanglard, D., Kuchler K, Ischer F, Pagani JL, Monod M, Bille J. Mechanisms of resistance to azole antifungal agents in Candida albicans isolates from AIDS patients involve specific multidrug transporters. Antimicrobial Agents Chemotherapy, 1995. 39(11):2378–2386.
[111] Cowen, L. E. The evolution of fungal drug resistance: modulating the trajectory from genotype to phenotype. Nature Review Microbiology. 2008. 6(3):187–198.
[112] Zhang, YQ, Gamarra S, Garcia-Effron G, Park S, Perlin DS, Rao R. Requirement for ergosterol in v-atpase function underlies antifungal activity of azole drugs. PLoS Pathogene, 2010. 6(6): e1000939.
[113] White TC, Marr KA, Bowden RA. Clinical, cellular, and molecular factors that contribute to antifungal drug resistance. Clinical Microbiology Review. 1998. 11(2):382–402.
[114] Shapiro RS, Robbins N, Cowen LE. Regulatory Circuitry Governing Fungal Development, Drug Resistance, and Disease. Microbiology And Molecular Biology Reviews, 2011. 75(2):213–267.
[115] Cowen LE, Steinbach WJ. Stress, drugs, and evolution: the role of cellular signaling in fungal drug resistance. Eukaryotic Cell, 2008. 7:747– 764.
[116] Anderson, J. B. Evolution of antifungal-drug resistance: mechanisms and pathogen fitness. Nature Review Microbiology, 2005 3:547–556.
[117] Mansfield BE, Oltean HN, Oliver BG, Hoot SJ, Leyde SE, Hedstrom L, White TC. Azole drugs are imported by facilitated diffusion in Candida albicans and other pathogenic fungi. PLoS Pathogene, 2010. 6(9):e1001126.
[118] Lupetti A, Danesi R, Campa M, Del Tacca M, Kelly S. Molecular basis of resistance to azole antifungals. Trends in Molecular Medicine. 2002. 8(2):76–81.
[119] Henry, K W, Nickels J T, Edlind T D. Upregulation of ERG Genes in Candida Species by Azoles and Other Sterol Biosynthesis Inhibitors. Antimicrobial Agents and Chemotherapy, 2000. 44(10):2693–2700.
[120] Morschhauser, J. Regulation of multidrug resistance in pathogenic fungi. Fungal Genetics and Biology, 2010. 47(2):94–106.
[121] Levin, DE. Cell wall integrity signaling in Saccharomyces cerevisiae. Microbiology and Molecular Biology Reviews, 2005. 69(2):262–291.
[122] Mitchell TG, Perfect JR. Cryptococcosis in the AIDS era-100 years after the discovery of Cryptococcus neoformans. Clinical Microbioogyl Reviews. 1995. 8:515–548.

[123] Park BJ, Wannemuehler KA, Marston BJ, Govender N, Pappas PG, Chiller TM. Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. Aids. 2009. 23: 525–530.
[124] Pfaller MA, Messer SA, Boyken L, Rice C, Tendolkar S, Hollis RJ, Diekema DJ. Global trends in the antifungal susceptibility of Cryptococcus neoformans (1990 to 2004). Journal of Clinical Microbiology. 2005. 43:2163–2167.
[125] Wertheimer AI, Santella TM, Lauver HJ. Successful public/private donation programs: a review of the Diflucan Partnership Program in South Africa. Journal of the International Association of Physicians in AIDS Care (Chic Ill) 2004; 3:74–9. 84–5.
[126] A. J. Carrillo-Muñoz, G. Giusiano, P. A. Ezkurra, and G. Quindós, "Antifungal agents: mode of action in yeast cells," Revista Espanola de Quimioterapia, 2006. vol. 19, no. 2, pp. 130– 139.
[127] Vanden Bossche H., Marichal P, Gorrens J, Bellens D, Moereels H, Janssen PAJ, Mutation in cytochrome P-450-dependent 14α-demethylase results in decreased affinity for azole antifungals. Biochemical Society Transactions, 1990.vol. 18, no. 1, pp. 56–59.
[128] Chang YC, Bien CM, Lee H, Espenshade PJ, Kwon-Chung KJ. Sre1p, a regulator of oxygen sensing and sterol homeostasis, is required for virulence in Cryptococcus neoformans. Molecular Microbiology. 2007; 64:614–629.
[129] Chang, YC, Ingavale SS, Bien C, Espenshade P, Kwon-Chung KJ. Conservation of the sterol regulatory element-binding protein pathway and its pathobiological importance in Cryptococcus neoformans. Eukaryotic Cell. 2009. 8:1770–1779.
[130] Ostrosky-Zeichner L, Casadevall A, Galgiani JN, Odds FC, Rex JH. An insight into the antifungal pipeline: selected new molecules and beyond. Nature Reviews Drug Discovery. 2010. 9:719–727.
[131] Ellis D. Amphotericin B: spectrum and resistance. Journal of Antimicrobial Chemotherapy. 2002. 49. Suppl.S1, 7-10.
[132] Vanden Bossche H, Marichal P, Odds FC. Molecular mechanisms of drug resistance in fungi. Trends inMicrobiology, 1994. vol. 2, no. 10, pp. 393–400.
[133] Kelly S L, Lamb D C, Taylor M, Corran A J, Baldwin B C, Powderly W G. Resistance to amphotericin B associated with defective sterol delta 8–7 isomerase in a Cryptococcus neoformans strain from an AIDS patient. FEMS Microbiology Letters. 1994, 122:39–42.
[134] Patel R. Antifungal agents. Part I. Amphotericin B preparations and flucytosine Mayo Clinic Proceedings, 1998. vol. 73, no. 12, pp. 1205–1225.
[135] Song M, Lee J, Kim MS, Yoon J, White TC, Floyd A, Heitman J, Strain AK, Nielsen JN, Nielsen K, Bahna Y. A flucytosine-responsive Mbp1/Swi4-like protein, Mbs1, plays pleiotropic roles in antifungal drug resistance, stress response, and virulence of Cryptococcus neoformans. Eukaryotic Cell, 2011. January 2012 vol. 11, no. 1, p. 53–67.
[136] Francis P, Walsh TJ. Evolving role of flucytosine in immunocompromised patients: new insights into safety, pharmacokinetics, and antifungal therapy. Clinical Infectious Diseases, 1992, vol. 15, no. 6, pp. 1003–1018.

