Antimicrobial resistance of infectious diseases of global importance: tuberculosis and malaria

B.E. Rivera-Chavira, **J.R. Adame-Gallegos** and **G.V. Nevárez-Moorillón**

Universidad Autónoma de Chihuahua. Facultad de Ciencias Químicas. Circuito Universitario S/N. Chihuahua, Chihuahua 31125, Mexico

The discovery of antibiotics was a breaking scientific advance in the fight against infectious diseases, and brought hope for the control of one of the most devastating human health problems. However, the versatility of microorganisms for environmental adaptation, promoted the presence of antibiotic resistant strains in just few years. The search for new antibiotics and the adaptation of the microorganisms to those new attacks has become an endless battle that we might eventually loose. Among infectious diseases of global importance, tuberculosis and malaria are examples of the major problem of antimicrobial resistance, since there are only few therapeutic schemes and few effective antibiotics. The aim of this chapter is to revise the mechanisms of antimicrobial resistance of pathogenic strains of *Mycobacterium tuberculosis* and *Plasmodium falciparum*.

Keywords: antimicrobial resistance; tuberculosis; malaria; mutations

1. General remarks

One of the most important advances of science in health-related issues was the discovery of antibiotics, and their massive use to treat severe infectious diseases; one of the consequences has been the increase and life expectancy and the increase in worldwide population. Although the antibiotic era initiated during the last century with the discovery of penicillin, the use of molecules with antimicrobial activity is no longer restricted to metabolites produced by an organism that had a lethal action against another organism. Today, the definition of antibiotic is related to the biological activity of an organic compound to inhibit or kill a microorganism; the activity is achieved by interaction of the antibiotic with specific bacterial targets [1]. In this regard, the source of the organic compound is not considered in the definition, and chemically synthetize compounds are also considered antibiotics. According to the organisms that are susceptible, antibiotics can present antibacterial, antifungal, antiparasitic, antiviral and even carcinogenic effects.

Antibiotics can be classified according to their mechanism of action and the biological target that they affect, including enzymatic activity or cellular structures, such as the cell wall, cellular membrane or ribosomes. Soon after the use of antibiotics to treat bacterial infections, and following the rules of preservation and survival, microorganisms started to develop mechanisms of control against the antibiotics present in their environments. After World War II, penicillin started to be used for the general population to treat infections caused by *Staphylococcus aureus* and in few years, penicillin resistant strains started to be isolated from clinical samples [2]. Even since, a race between the development of new antibiotics and the appearance of resistant microbial strains has pushed to the emergency of multi resistant strains, making some bacterial infections difficult to treat [1]. The presence of naturally antibiotic resistant strains such as *Pseudomonas aeruginosa* has posed the question of the existence of resistant mechanisms in nature, even before the antibiotic era. A recent study has described antibiotic resistance mechanisms in soil isolates that have not previously been exposed to antibiotics [3].

An emerging environmental problem is the persistence of antibiotics in the environment, once its intended use has finished. An orally ingested antibiotic has just a short lifespan in the organism, and is either partially transformed or discharged in its original form. The antibiotic is then released and accumulated in the environment, with consequences not yet fully understood [4]. The presence of antibiotics in water and soil can lead to the development of resistant strains in the environment, which can serve as antibiotic resistance genes reservoirs to be later transferred to pathogenic bacterial strains. The indiscriminated use of antibiotics in public health, but also in veterinary and even in agricultural uses also contributes to the emergency of resistant strains. The multi resistant strain phenomenon is still not fully understood and the consequences of the extensive use of antibiotics are just arising.

Even with all this problems, antibiotics continue to save lives around the world, and antibiotic therapy continues to be one of the major advances in clinical treatment of severe infectious diseases. Among infectious diseases of global importance, tuberculosis and malaria are examples of the antimicrobial resistance problem, since there are only few therapeutic schemes and few effective antibiotics. The special structural characteristics of *Mycobacterium tuberculosis* cells makes it difficult for many commonly used antibiotics to even cross the cell wall, so just few antibiotics are effective. On the other hand, there are just few proven antibiotics against eukaryotic parasites such as *Plasmodium falciparum*. The intracellular nature of these infections is another problem for antibiotic therapy that needs to be effective against the intracellular forms and also be able to cross the host cellular membranes to reach its target.

The emergency of resistance strains of *M. tuberculosis* and *P. falciparum* strains to the few therapeutic antibiotics in existence, has directed to the search of new effective antibiotic molecules, but also to the understanding of the mechanisms of control in the resistant strains; this information can be useful on the global control of the infections.

2. Tuberculosis

Human tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (MTb), which is an acid-alcohol-resistant bacterium that usually causes a pulmonary infection that can be disseminated to other organs (extrapulmonary Tb). Pulmonary tuberculosis symptoms include chronic coughing, phlegm production either with or without blood, weight loss, fever and nocturnal sweating [5]. Extrapulmonary Tb occurs in 10 to 42% of the patients, depending on the MTb strain, as well as on the age, ethnic group and immune system of the patient [6].

It is estimated that one third of the world population is infected with *M. tuberculosis* and its clinical importance has reemerged in part as being a disease associated with AIDS pandemia [7-8]. In 2013, a total of 9 million people were reported as being infected with tuberculosis, with 1.5 million deaths; 360,000 of them were also infected with HIV virus [9].

The transmission mechanism of tuberculosis is by aerial dispersion from saliva drops of patients with active infection, that are expelled when speaking coughing or sneezing. The development of the infectious disease depends on the infectious dose of the strain, as well as on the immune system of the patient; almost 90% of them have an asymptomatic and latent infection; 5% will develop a symptomatic disease within 18 months of infection and the other 5% will show symptoms later in their life [10]. Nearly 2 billion people worldwide have a latent tuberculosis infection that can be reactivated [9].

Effective control depends on the rapid detection of the pathogen and the strict adherence to a long antimicrobial treatment. Initial treatment consist of an intensive phase of two months using a combination of four antibiotics: isoniazid, rifampicin, ethambutol and pyrazinamide, identified as first line drugs; followed by a second phase of four months with isoniazid and rifampicin. Isoniazid and rifampicin are bactericidal, while pyrazinamide eliminated those microorganisms that are not in replication. Since the treatment is for an extended time period and there are severe secondary effects, patients often abandon the antimicrobial scheme, with the consequent presence of mycobacterial strains resistant to first line antifimic drugs (TB-MDR). This is known as acquired or secondary resistance, in contrast with primary resistance where a patient is infected with an originally resistant *M. tuberculosis* strain [11-12].

In 2014, WHO reported that 3.5% of new tuberculosis cases and 20.5% of cases with previous treatments, developed multidrug resistant tuberculosis (TB-MDR), meaning that the strains were resistant to isoniazid and rifampicin [9]. Treatment of TB-MDR infections can last two or more years and is based on the use of second line drugs such as fluoroquinolones and some intravenous aminoglycosides such as kanamycin, amikacin or capreomycin. Treatment is more aggressive and costly than primary treatment [13].

The presence of MTb cases that are resistant to isoniazid and rifampicin, as well as resistant to one fluoroquinolone and to an injectable aminoglycoside has been reported in at least 100 countries, and has been given the notation of extremely resistant (TB-XDR) strains. Approximately 9% of the patients infected with TB-MDR strains are TB-XDR and those cases have very poor therapeutic options, with poor prognosis [9, 13-14].

3. Mechanisms of resistance in tuberculosis strains

Molecular basis of drug resistance is a fundamental knowledge in the control of antibiotic resistance pathogens. In the case of tuberculosis, the rich composition of mycolic acids on the mycobacterial cell wall confers a natural resistance, due to the low permeability to most antibiotics; but also the action of efflux transporters and to the presence of chromosomal resistance genes are responsible for antibiotic resistance mechanisms. Mutations on different chromosomal genes have been associated with resistance to rifampicin, isoniazid, streptomycin, pyrazinamide, fluoriquinolones, aminoglycosids or cyclic peptides [15-16].

3.1 Rifampicin resistance

Rifampicin is a lipophilic rifamicin, with an aminomethilpiperacin group on the 3 position; it is a semisynthetic antimicrobial compound that inhibits the synthesis of mRNA by binding to the RNA polymerase. It has a bactericidal effect on active MTb infection; and it also attacks cells under latency. Resistance to this antibiotic is due to mutations in the rpoB gene (3534 bp in size), that codifies for the β subunit of the RNA polymerase, the site of union of the antibiotic [17]. Mutations in the gene generate conformational changes in the subunit, which reduces its affinity for the rifampicin. In 96% of the strains, the mutation responsible for the resistance is an 81 bp region (27 codons) known as rifampicin resistant determinant region (RRDR) [17-18]. Most common mutations have been found in codon 531, where there is a change of serine for leucine (TCG-TTG) or threonine (AGC-ACA). Another frequent mutation is in codon 526. Almost all rifampicin resistant strains are also resistant to isoniazid, and the simultaneous resistance to both antibiotics makes them multi resistant tuberculosis strains (TB-MDR) [19-20].

3.2 Isoniazid resistance

Isoniazid (INH: isonicotinic acid hydrazide) has a simple structure that contains a pyridine ring and a hydrazide group and both groups are important for the high activity against *M. tuberculosis* (Fig. 1a) [21]. It is a synthetic prodrug that when it is activated *in vivo*, can oxidize or acylate protein groups that are part of the synthesis of mycolic acids, which are found in the *M. tuberculosis* cell wall [22]. Activation of isoniazid is carried out by a catalase-peroxidase enzyme codified by the *katG* gene; once activated, the drug interferes with the elongation of fatty acids during the synthesis of mycolic acids, by inhibition of the NADH dependent enaoil-ACP reductase that is codified by the *inhA* gene. Activation of IHN produces oxygen reactive compounds that attack multiple targets in the mycobacterial cell [23].

Mutations on *katG* gene and on the promoting region of the *inhA* gene are associated with 70-80% of the *M. tuberculosis* isolates that are resistant to isoniazid; however, there are other genes with mutations, such as *kasA, ndh* and the intergenic region of the *oxyR/ahpC* complex that are also responsible for isoniazid resistance [24-27]. The size of the *katG* gene is of 1771 bp, but 50-95% of the isoniazid resistant strains of *M. tuberculosis* carry the mutation KatG S315T (codon 315 Ser changes to Thr, Asn or Arg) [28].

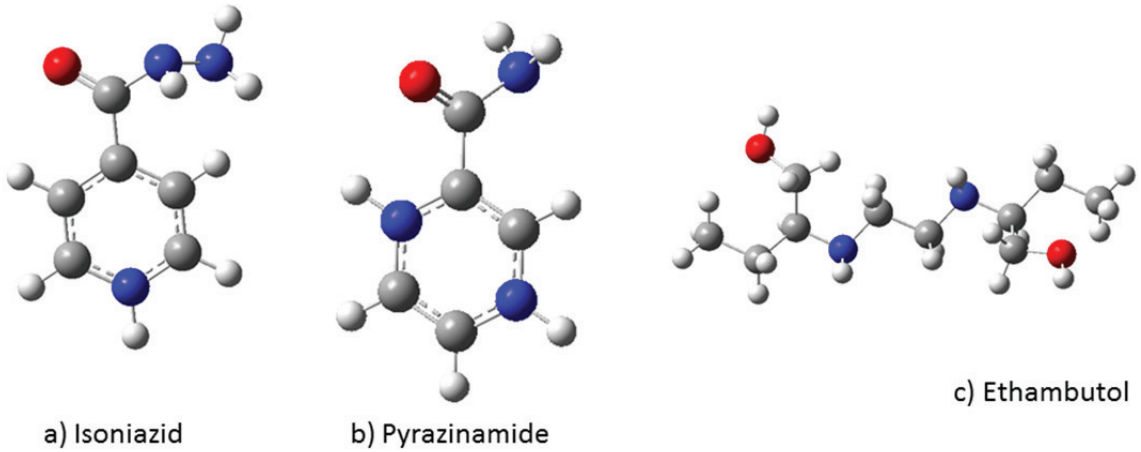

Fig. 1 Molecular structure of some therapeutic drugs used to treat tuberculosis.

3.3 Pyrazinamide resistance

Pyrazinamide (PZA) is a synthetic derivate of the nicotinamide; its use can reduce tuberculosis treatment from 9-12 to 6 months. It can inhibits the growth of bacterial cells in dormancy stage that are found in acidic environments, and as such, it has an sterilizing effect on latent *M. tuberculosis* cells that reside inside macrophages and that are not affected by other antibiotics. This drug is highly specific, since it does not affect *M. bovis* strains, due to a mutation in *pncA* gene in this microorganism [29-30].

Pyrazinamide is a prodrug that is converted into its active form, pyrazinoic acid by the pyracinamidase mycobacterial enzyme (Fig. 1b). The drug can passively diffuse into the macrophages, where is converted into its active form and is intracellularly accumulated by a deficient transmembrane transport system. The pyrazinoic acid acts on the pyracinadimase/nicotinamidase enzyme, which is codified by the *pcnA* gene. Most of the mutations responsible for pyrazinamide resistance occur in a 561 bp region of the *pcnA* gene or on an 82 bp region on the promotor [30-31].

3.4 Ethambutol resistance

Ethambutol (EMB: 2-[2-(1-hydroxybutan-2-ylamino)ethylamino]butan-1-ol) is a bacteriostatic compound that is active only towards mycobacterial cells that are actively multiplying (Fig 1c). Ethambutol interferes with the biosynthesis of the arabinogalactan present in the mycobacterial cell wall, carried out by the arabinosiltransferase enzyme, codified by the *embB* gene, found in the *embCAB* operon [32].

Close to 50% of the *M. tuberculosis* strains that show resistance to ethambutol have mutations on the *embB* gene. A punctual mutation has been more frequently reported on the 306Met codon, with a substitution for Val, Leu or Ile [33-34].

3.5 Fluoroquinolones resistance

Fluoroquinolones derived from quinolones, are used in the treatment of *M. tuberculosis* primary drugs resistant infections and has a bactericidal effect. It bounds to the type II topoisomerase DNA gyrase that inhibits DNA supercoilling, and its action causes microbial death [35-36]. DNA gyrase is formed by two subunits (A and B) codified by *gyrA* and *gyrB* respectively [37].

Flouroquinolone resistance has been associated with mutations in the hypervariable regions of 320 and 375 bp on the *gyrA* and *gyrB* genes that codifies for the DNA gyrase. Nearly 80% of the mutations have been located in *gyrA* gene [38]. Mutations in the *gyrA* gene related to resistance of fluoroquinolones are associated with substitutions of the amino acids 89, 90, 91 or 94 of the gyrA subunit. Those mutations are found in 50-90% of the highly resistant strains. Aminoacid substitutions in gyrB subunit usually confer low levels of resistance [39].

3.6 Kanamycin, Amikacin and Capreomycin resistance

Kanamycin (KAN) and Amikacin (AMK) are aminoglycoside antibiotics, while Capreomycin (CAP) is a cyclic peptide. Even when the compounds are of two different chemical families, their antimicrobial activity is related to inhibition of protein synthesis. Amikacin and kanamycin inhibit protein synthesis by union to the 16S rRNA in the 30S ribosomal subunit [40]. In tuberculosis, the mechanism of action of capreomycin is not well defined, but it has been reported that interferes with DNA translation and inhibits phenylalanine synthesis [41].

M. tuberculosis strains with high level of resistance to kanamycin and amikacin has been associated with a mutation in A1401G of the *rrs* gen that codifies for the 16S rRNA [42]. Antibiotic resistance to capreomycin has been associated with mutations in the *tlyA* gene that codifies for an rRNA methyl transferase, which is specific for the rRNA 2'-O-methylation; therefore, mutants are usually deficient in rRNA methylation [43]. In a recent study, 80% of the clinical isolates with low levels of kanamycin resistance presented mutations in the promoter of *eis* gene in M. *tuberculosis* that codifies for an aminoglycoside acetyltransferase [44].

There are a large numbers of reports on mutations in the target regions of genes associated with tuberculosis drug resistance, but there are also reports on mutations found in other regions that are also associated with *M. tuberculosis* resistant strains. On the other hand, mutations on the target regions have showed not to be associated with resistance, suggesting that in those strains, drug resistance can be related to other mechanisms.

4. Malaria

Together with HIV/AIDS and tuberculosis, malaria affects mostly populations found in developing countries. Malaria as we know it may be have been described in the 18th Century, although Indian and Brahmanic scriptures had references to "the fever demon" Takman, and the characteristic fever presented every 48 to 72 hours; along with Sumerian, Egyptian, and Greek texts, all have reference to the disease in Mediterranean Europe [45]. In 2010, Hawass and collaborators identified one of the etiological agents that cause the disease *Plasmodium falciparum* in the mummy of Tutankhamun, the famous Egyptyan pharaoh [46]. Back in 1880, the *Plasmodium* parasite was discovered by Laveran and in 1897, Ronald Ross described its transmission by *Anopheline* vectors [47]. Very broadly, this disease can be classified in tertian and quartian malaria, The first, caused by *P. vivax*, *P. ovale*, and *P. falciparum* with febrile paroxysms every 48 hours, whereas in the latter, the febrile paroxysms occur every 72 hours and is caused by *P. malariae* [48].

Common challenges of the 21st Century in the treatment of malaria are associated with social events such as wars, displacements, migrations, population increase, as well as environmental and climate changes [49]. To this matter, it must be taken into account that potential outbreaks of malaria unrelated infections, such as the recent Ebola outbreak in West Africa, can hamper malaria control programs [50].

Previous global efforts to eradicate the vector with dichloro-diphenyl-trichloroethane (DDT) in the mid 20th Century have proved that were not a long-term solution. Resistance to accessible drugs like chloroquine and sulphadoxine/pyrimethamine in endemic areas has also complicated matters. Since the 17th Century, the recurrent use of quinine has derived in major focal points of *P. falciparum* resistance, especially in sub-Saharan Africa. In the latter quarter of the Century, only 4 new anti-malarial drugs were developed, representing 0.3% of all new drugs approved for use at that time [51]. In this sense, there is a continuous interest on carrying out research on plants and trees used with therapeutic purposes, to identify new compounds with biological activity [52-53].

Natural resistance or immunity to malaria, although it may not be completely protective, occurs after many years of recurrent infections [54], where antibodies play a crucial part, especially IgG [55-56]. Several compounds have been targeted as vaccine candidates, either on the pre-erythrocytic stage, during sporozoite invasion and liver stage, as well as on erythrocytic stages, and in the mosquito [57] with little effectiveness so far on first generation-vaccines [58]. Throughout history, an alternative way of developing resistance to the disease has not been associated with the parasite, its vector or drugs, but with human evolution [45]. One of the most notorious cases is the Duffy negativity of red blood cells (rbc) from indigenous population of Africa, who cannot develop *P. vivax* malaria, due to the absence of the Duffy antigen required for the merozoites to invade the rbc [59]. Other blood-related diseases such as thalasemmias, Glucose-6-Phosphate Dehydrogenase Deficiency (G6PD), and sickle cell trait, are mutations associated with Haldane's "malaria hypothesis" back in the 1940s, where is proposed that these polymorphisms are a consequence of evolutionary strategies to protect humans from malaria [45].

Together with these immunological-based efforts, the need for new potential antimalarial drugs appears to be a priority in the field. Multiple parasite mutations in drug-resistant genes have been observed, decreasing the effectiveness of the current available compounds that includes quinine, artemisinin, and antifolate drugs [60-61].

Despite the fact that there are only four known species of *Plasmodium* that infect humans, being *P. falciparum* that is most important, an increase in the number of patients can be due to changes in domestic habits, climate and microclimate changes, migrations and social disturbances. Drug resistance to malaria is associated to a wide range of social, genetic, and epidemiological factors, such as polymorphisms in the parasite/host and the inappropriate use of current anti-malarial drugs [62].

5. *P. falciparum* drug resistant strains

The main obstacle on the war against malaria is the appearance of drug resistant parasite strains that is defined as the capacity of the parasite to be able to survive or reproduce, even when the amount of administrated drug reaches the infected erythrocyte or the parasite, for the time required for its action. The development of resistance to drugs depends on environmental and biological factors, as well as on the selective pressure given by the presence of malaria drugs in the patient [63]. Intracellular targets of malaria drugs are the digestive vacuole (DV), as well as the cytosol, mitochondria, apicoplast and the parasite membrane [64]. Drug resistance promotes the production of gametocytes that are the responsible for the transmission of the resistant genotype [65]. There are two processes for the presence of drug resistance in malaria parasites: i) as a result of drug use by an individual and the appearance of *de novo* mutations and ii) dissemination of resistance genes to other parasites [66].

Anti-malaria first-line drugs: cloroquine (CQ) sulfadoxine/pyrimethamine (SP) and artemisinin are efficient in the elimination of *P. falciparum* infected erythrocytes (Figure 2). Resistance is usually due to punctual mutations, genetic duplication or alterations in multiple loci, with direct affection to metabolic pathways. For some of the drugs, a single mutation in the parasite is needed to present resistance, while for others multiple mutations are required [67].

Resistance to multiple malarial drugs can be due to punctual mutations on the genes that codifies for modifications on target molecules, with the consequent diminished drug affinity (pyrimethamine, cycloguanil, sulphonamide, atovaquone, artemisinin resistance); it is also related to changes on the accumulation of the drug or to efflux mechanisms that eliminate the drug from the specific site of action (chloroquine, amodiaquine, quinine, halofrantine, mefloquine resistance) [67-68]. Genes identified as responsible for drug resistance in *P. falciparum* are an dihydropteroate synthase (Pfdhps), an dihydrofolate reductase (Pfdhfr), the chloroquine resistance transporter (Pfcrt), the multidrug resistance 1 protein (Pfmdr1), the Na^+/H^+ exchanger (Pfnhe-1) and the cytochrome b [65].

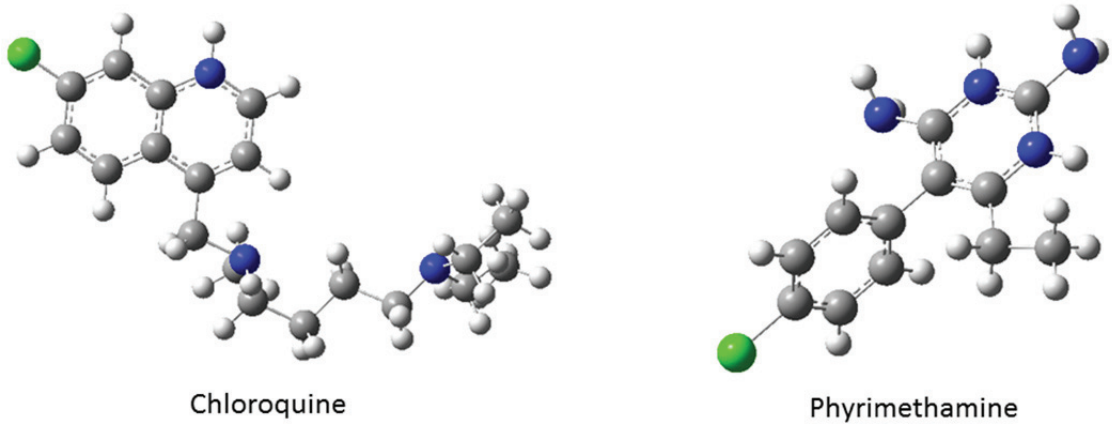

Fig. 2 Molecular structure of therapeutic drugs used to treat malaria.

5.1 Cloroquine resistance

The chloroquine (CQ) is a 4-aminoquinolone derivate from quinine (Figure 2) that adheres to the heme group of the hemoglobin in erythrocytes; in cells infected with *P. falciparum,* the hemoglobin molecule is not transformed to hemozoine, that is a non-toxic form for the parasite and that is accumulated in the parasite's digestive vacuole. Resistance mechanisms for chloroquine are associated with mutations in the *pfcrt* gene that codifies for the chloroquine resistance transporter (PfCRT) of *P. falciparum*. The most common substitution is a Lys 76 changed to Thr (K76T) and this is considered a marker for CQ treatment failure. However, there are several polymorphisms that generate PfCRT haploid mutants that are specific for a geographic region, while there is only one haplotype on wild type CQ sensible parasites [69-71]. The PfCRT transporter is located in the digestive vacuole membrane and its function appears to be related to maintaining an osmotic balance by the transportation of ions and the degradation of hemoglobin products [72]. Resistance is associated with an increase in chloroquine efflux from the digestive vacuole by a mutated transporter. Two mechanisms have been proposed: i) the elimination of the diprotonated chloroquine by an

electrochemical gradient and ii) diprotenated cloroquine elimination coupled to energy production; in both cases, chloroquine concentration in the vacuole is reduced [73].

The gene for the transporter pfmdr1 that codifies for an homologue1 P-glycoprotein (PfPgH-1), also known as (PfMDR1) presents polymorphism in *P. falciparum* that have been associated with response to other antimalarial drug (amodiaquine and monodesethylamodiaquine, its main metabolite). It has also been associated with chemical compounds derivate from artemisinin; resistance to amodiaquine is also related to mutations on the CQ (pfcrt) transporter gene [70, 74-75].

Another effective drug against the *P. falciparum* is quinine (QN); resistance is not widely examined since the mechanism is related to multiple genes. Sodium hydrogen ion exchanger gene (pfnhe1) is associated with resistance to QN and is located in the chromosome [76].

5.2 Pyrimethamine and sulfadoxine resistance

The combination pyrimethamine/sulfadoxine (SP), acts in a synergic way to inhibit the growth of *P. falciparum* by suppressing folate synthesis in the parasite. The dihydrofolate reductase (DHFR) is codified by the *dhfr* gene and catalyses the reduction of dihydrofolate to tetrahydrofolate, an essential cofactor for the synthesis of nucleic acids and methionine. The most common mutations associated with pyrimethamine resistance include A16V, N51I, C59R, S108N and I164L mutations of the DHFR [77-78]. The sulfadoxine is a potent inhibitor of the dihydropteroate synthase (DHPS), which is codified by the *pfdhps* gene; the most common mutations reported are S436A, A437G, K540E, A581G y A613S/T [77, 79]. In both cases, mutations structurally modified the active site of the enzyme so that the drug cannot longer be bounded and the parasite becomes resistant [80].

5.3 Artemisin resistance

Artemisinin (ART) is a sesquipene lactone with an endoperoxide group and is an important drug used in malaria treatment, with synthetic derivatives that are even more potent, such as arthemether, artemotil and artesunate. These compounds are an important alternative for malaria treatment, given the increasing resistance of the parasite to drugs such as chloroquinone and pyrimethamine/sulfadoxine [81].

Artemisin derivatives are characterized for their rapid action, they are safe and act at different stages in the life cycle of *P. falciparum*. They have the capacity to kill gametocytes with the consequent reduction of malaria transmission [82]. The rapid recovery promotes the abandonment of the treatment, causing the appearance of resistance strains from the parasites still living inside the patient [83-84]. On the other hand, a therapy with only one drug increases the appearance of mutations; therefore in 2001, WHO established the use of fixed doses of malaria drugs, in the artemisinin-based combination therapies (ACTs) program (including artemether-lumefantrine, artesunatemefloquine, and artemether-amodiaquine) as the first-line treatment of malaria caused by *P. falciparum*. The ACT program is based on the combination of artemisinin (that is transformed *in vivo* into the more potent dihydroartemisinin drug) that has a short half-life span, with drugs that have a longer half-life time period; this combination can eliminate 99.99% of the parasites [85-86]. Even with the efforts done worldwide to control malaria, there are already reports of artemisinin resistant strains of *P. falciparum* in Asia [87]. There are several model of mode of action of artemisinin, and there are also suggestions on mutations that are related to resistance of the parasite to the drug, but their role in resistance is not yet completed linked [73].

6. The road ahead

For thousands of years, malaria and tuberculosis have had a direct impact in human population. Even in the antibiotic era, these infectious diseases have caused many deaths worldwide and are still a major problem in developed and undeveloped countries. The search of new antimicrobials, the development of synthetic drugs, the study of remedies used by native groups, are all possible paths to a better control of the disease. Educations on health care and basic sanitary measures are important for the control of transmissible diseases as well.

The knowledge of the mechanisms of antimicrobial resistance developed by pathogenic strains, can lead to the description of new therapeutic strategies and diagnostic assays, which can allow us to better control the dissemination of these diseases and the rapid recovery of the patients. Multidisciplinary research of biological disciplines such as ecology, epidemiology, and genetics must be met in an attempt to understand and prevent further cases of drug resistance.

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