European Journal of Therapeutics

DOI: 10.5152/EurJTher.2019.18075

Review

Drug Resistance in Parasitic Diseases

Hatice Ertabaklar, Erdoğan Malatyalı, Sema Ertuğ

Department of Parasitology, Adnan Menderes University School of Medicine, Aydın, Turkey

ABSTRACT

Owing to the lack of or the ineffectiveness of vaccines for life-threatening parasitic diseases, chemotherapy is the current strategy to prevent parasitic diseases. Drug resistance disrupts chemotherapeutic options, thereby increasing the need for novel drugs in parasitological treatments. The most common resistance mechanisms are decreased drug uptake, export of drugs from parasites, genetic modifications, loss of drug activity, and alteration of the drug target. Drug resistance mechanisms should be well defined to develop new strategies to control parasitic diseases. This measure will ensure new effective treatment options for clinicians. In the recent years, isolation and characterization of resistance-related genes and proteins has considerably increased our knowledge. This review mostly focuses on new studies and common parasitic diseases.

Keywords: Chemotherapy, drug resistance, parasitic diseases

INTRODUCTION

Globally, parasitic diseases have immense health, social, and economic impacts, especially in tropical countries. Protozoan and helminthic diseases (mostly malaria and schistosomiasis) have resulted in almost 1.1 million deaths. Moreover, globally distributed protozoan parasites lead to high disability-adjusted life years (1). Owing to the lack of licensed vaccines and effective drugs, the burden of parasitic diseases rises regularly. Moreover, drug resistance is a threat in regions where appropriate medication is available. Accordingly, there exists an emerging need for novel drugs for the effective treatment of protozoal diseases, particularly for malaria, toxoplasmosis, and leishmaniasis (2).

Malaria

Malaria is one of the most common protozoan infections with a high morbidity and mortality rate. This human disease is caused by five *Plasmodium* species (*P. falciparum, P. vivax, P. ovale, P. malariae* and *P. knowlesi*). According to the World Health Organization report in 2018, approximately 3.2 billion people were under the risk of malaria, 198 million were infected, and 584.000 deaths were reported with malaria globally (3). Antimalarial drugs are mainly divided into three groups according to their mechanisms of action: quinolone, antifolates, and artemisinin derivatives.

Globally, antimalarial drug resistance to *P. falciparum, P. vivax,* and *P. malariae* have been reported; numerous researches have highlighted this topic. Chloroquine (CQ) resistance spread in Africa, thereby causing a 2-3 fold increase in malaria-related deaths in the 1980s. The resistance in *P. falciparum* was characterized by a mutation on CQ resistance carrier (Pfcrt) gene, localized in a 36 kb segment on chromosome 7 (4). After this dramatic increase, sulphadoxine/pyrimethamine (SP) became the first choice antimalarial drug instead of CQ treatment. At the beginning of 2000, the parasite improved SP resistance. Accordingly, combination therapy regimens were applied to increase drug efficacy and slow the development of drug resistance (5). Recently, artemisinin-based combination therapies are effectively used to treat malaria. However, artemisinin resistance has appeared in Southeast Asia, leading to a global risk for malaria treatment and control (6).

Antifolate drugs inhibit *P. falciparum* dihydropteroate synthase (Pfdhps) and dihydrofolate reductase-thymidylate synthase (Pf-dhfr-ts) enzymes, which are essential for folate biosynthesis. Biochemical and genetic studies on *P. falciparum* have claimed that the mutations in the aforementioned genes reduced the drug sensitivity of antifolates. Whole-genome sequencing of an artemisinin-resistant parasite revealed that mutation in artemisinin resistance was associated with kelch 13 (K13) protein in clinical and field isolates of *P. falciparum* (7). The *P. falciparum* multidrug resistance protein 1 gene (Pfmdr1) is located on chromosome 5, which has a single exon. This protein is similar to PfCRT protein; it is found in the digestive vacuole of the parasite and acts as a basis for adenosine triphosphate (ATP) binding. The N86Y, Y184F, S1034C, N1042D, and D1246Y mutations in Pfmdr1 gene help detect the drug sensitivity of a variety of drugs such as CQ, quinine, mefloquine (MQ), halofantrine, lumefantrine, and artemisinin. Among these, N86Y and N1042D mutations are associated with resistance. The K76T and A220S mutations in the Pfcrt gene and the N86Y mutations in the Pfmdr1 gene are associated with high resistance

This manuscript is an activity of Society for Clinical Microbiologists of Turkey (KLİMUD), Medical Parasitology Study Group.

How to cite: Ertabaklar H, Malatyalı E, Ertuğ S. Drug Resistance in Parasitic Diseases. Eur J Ther 2020; 26(1): 1–5.

ORCID IDs of the authors: H.E. 0000–0001–7997–6433; E.M. 0000–0002–3943–467X; S.E. 0000–0002–6448–7482

Corresponding Author: Hatice Ertabaklar **E–mail:** hatice@adu.edu.tr

Received: 10.12.2018 • **Accepted:** 12.02.2019

to CQ. In addition, the variation in the copy number of Pfmdr1 gene depends on the resistance levels of kinase, MQ, lumefantrine, halofantrine, and artemisinin (8). Another *P. falciparum* multidrug resistance-associated protein gene (Pfmrp) is located on chromosome 1 and has one exon; it belongs to the ATP-binding cassette carrier family and resembles the Pfmdr1 gene. This protein facilitates the transport of organic anionic substrates such as oxidized glutathione, glucuronate, sulfate conjugates as well as drug transport. Two mutations at positions Y191H and A437S in the Pfmrp gene were found to be associated with CQ and quinine resistance. On chromosome 13, *P. falciparum* has Pfnhe1 gene, which has two exons that encode sodium-hydrogen exchanger protein and is associated with resistance to quinine (9). *Plasmodium falciparum* bifunctional dihydrofolate reductase-thymidylate synthase gene (Pfdhfr-ts): Pyrimethamine resistance is mainly associated with the point mutation of the S108D codon in this gene; other mutations in the N51I, C59N, and 166L positions support resistance as well. *Plasmodium falciparum* dihydropteroate synthase gene (Pfdhps): Five mutations in the Pfdhps gene (S436A/F, A437G, L540E, A581G, and A613T/S) were reported to be associated with sulfadoxine resistance in *P. falciparum* (10).

Atovaquone, an antimalarial drug that binds to the ubiquinol binding site of cytochrome b (cytb), destroys the electrochemical potential of the mitochondrial membrane and is lethal to the parasite. The ubiquinol binding site is a highly conserved region; once mutated, it gives resistance to atovaquone. A single mutation in the Y268N/S/C codon in the cytb gene was associated with atovaquone resistance in *P. falciparum* isolates (11).

Increased chloroquine sensitivity in *P. vivax* is closely related to the Y976F mutation in the Pvmdr1 gene, a homologue of Pfmdr1 (12). Unlike *P. falciparum*, the Pvcrt gene, a homologue of the Pfcrt gene, is not associated with CQ resistance in *P. vivax*. The MQ resistance in *P. vivax* is associated with the amplification of the Pvmdr1 gene. In addition, in vitro studies have revealed that Y976F mutation in Pvmdr1 gene was associated with resistance to MQ and artesunate. However, further clinical trials are needed in this case. The point mutation in the codon F57L/ I, S58R, T61M, and S117T/N of the Pvdhfr gene has been reported to be associated with pyrimethamine resistance and treatment failure in *P. vivax* (13).

Toxoplasmosis

Toxoplasma gondii (*T. gondii*), the causative agent of toxoplasmosis, is an intracellular parasite infecting humans and a wide variety of vertebrates. Infection is usually asymptomatic; however, immunosuppression and congenital can lead to life-threatening outcomes in infants and congenitally infected fetuses in pregnancy. Three main clones of *T. gondii* have been identified: Type I (RH etc., highly virulent), Type II (ME-49 and PRU etc., avirulent), and Type III (NED etc., avirulent) (14). Sulfonamide and pyrimethamine are commonly used drugs to treat toxoplasmosis. They have synergistic effects in inhibiting *T. gondii* replication by sequentially inhibiting parasite dihydropteroate synthase (dhps) and dihydrofolate reductase (dhfr). These two enzymes prevent the synthesis of the folate compounds required for the survival and replication of parasite. However, numerous treatment failures have been reported in toxoplasmic encephalitis, chorioretinitis, and congenital infection. Some failures may be associated with drug intolerance, malabsorption, and/or drug resistance (15). In a study, common anti *T. gondii* drugs were tested in vitro on 17 different strains: sulfadiazine (SDZ), atovaquone, and pyrimethamine. Despite some differences, no resistance to pyrimethamine and atovaquone was detected; however, resistance to SDZ was detected in three strains (16). The amino acid mutations in dhps result in resistance to sulfonamides and sulfones. Antifolate resistance due to the point mutations in dhps and dhfr coding genes was reported in *P. falciparum*. Pyrimethamine resistance was linked to a mutation in the dhfr enzyme (Ser-108 Asn 108) and other mutations (N51I, C59R, I164L, and A16V). Resistance to sulfonamides and sulfones were due to the amino acid mutations in dhps at five positions (S436A/F, A437G, K540E, A581G, and A613/T) (17). Aspinall et al. (18) revealed six mutations at positions 407, 474, 560, 580, 597, and 627 in the dhps gene of *T. gondii*. In sulfonamide resistance, only one mutation (at 407) was reported to be equivalent to *Plasmodium* species (at 437). This mutation has also been detected in a sulfamethoxazole-resistant strain, which has been rendered resistant in the laboratory. In a study among five *T. gondii* isolates from congenital toxoplasmosis, dhps was compared between those and previous isolates. Four isolates have been reported to be resistant to SDZ. Nineteen polymorphisms were detected in the exon of the dhps gene, and four were detected for the first time in this study. However, no relationship exists between SDZ susceptibility and gene polymorphism (15). In a study conducted in 2017, a previously unidentified mitochondrial protein (TgPRELID) was identified in *T. gondii* and associated with multiple drug resistance. Furthermore, the study reported that the mechanism of resistance was necessary to investigate (19).

Main Points:

- Drug resistance in life-threating parasitic diseases is a major problem.
- The main mechanism of resistance are drug uptake, export of drugs from parasites, genetic modifications, loss of drug activity, and alteration of the drug target.
- New strategies to control parasitic diseases are necessary.
- The contribution of genetic studies to drug resistance is very precious.

Leishmaniasis

Leishmaniasis is a vector-borne infectious disease with a zoonotic/anthropic character, spreading worldwide except Antarctica. The disease has different forms: cutaneous (CL)/mucocutaneous leishmaniasis are relatively less important, non-lethal, and self-healing skin infections; visceral leishmaniasis (also known as Kala-azar) is a systemic infection that effect viscera and cause deaths of people in epidemics. Almost 12 million people in 98 countries have been infected with *Leishmania* species, and 350 million people live in risky regions (20). In Turkey, approximately 2000 leishmaniasis cases are reported annually.

Sodium stibogluconate (Pentostam®) and meglumine antimoniate (Glucantime®) are used as the first choice in the treatment of leishmaniasis for more than 50 years. In recent years, resistance has appeared in South America, Europe, and the Middle

East, and India in particular. Treatment with pentavalent antimony compounds in Bihar, a leishmaniasis endemic region in India, has failed in 60% of the cases. Alternatively, few drugs that can be used include amphotericin B, pentamidine, and oral miltefosine. Reduced efficacy of miltefosine and resistant cases of meglumine antimoniate in the treatment of CL in the Middle East has been reported (21). The presence of drug-resistant leishmaniasis cases has also been reported in Turkey from Urfa, Hatay, Diyarbakir, and Aydin (unpublished data).

Resistance mechanisms in leishmaniasis have been better understood through molecular studies in recent years. The ubiquitin and amino acid permease (AAP3) have been reported to play a role in resistance in *L. tropica* isolates (22). In addition, another study found five genes that could play a role in resistance: aquaglyceroporin (AQP1) and ATP-binding cassette transporter (abc-3) that play a role in drug release; phosphoglycerate kinase (PGK) that plays a role in glycolysis metabolism; and mitogen-activated protein kinase (MAPK) and protein tyrosine phosphatase (PTP) responsible for the phosphorylation pathway. In resistant isolates, three of these genes (multidrug resistance protein A, PTP, and PGK) were reported to be upregulated and the other two (AQP1 and MAPK) were downregulated (23). MAPK1 (Ld-MAPK1) is associated with antimony resistance in *L. donovani*. Moreover, *L. major* MAP2 antimony resistance is regulated by phosphorylating influx pump AQ120. In another study, increased abc-3 and decreased AQP1 gene expression were shown in laboratory-derived Sb-resistant *L. panamanensis* isolates. However, it was not significant in clinical isolates in which abc-2 was significantly higher. Laboratory and clinical Sb-sensitive/resistant *L. panamanensis* isolates were significantly increased mt2a (xenobiotic scavenging) expression in Sb-sensitive isolates in different types of macrophages. Thus, gene expression was associated with drug transport, and metabolism in parasite-infected cells might also be important in resistance and susceptibility to *Leishmania spp*. (24). Antimony resistance mechanisms are usually studied experimentally in Leishmania because of the intracellular location of the parasite. Therefore, studying the effectiveness of drugs is difficult due to the release of the drug into the host cell and the interference of the drug in the cell compartments.

Giardiasis

Giardia intestinalis (*G. intestinalis, G.lambia*) is a microaerophilic protozoon found in the gastrointestinal tract of humans and an important cause of steatorrhea with an incidence of 200–300 million cases and an estimated prevalence of 1 billion (25). It is one of the most common intestinal parasites in our country. Depending on the genotype and drug resistance of the parasite, acute or chronic disease can develop. Symptoms include nausea, swelling, diarrhoea, vomiting, dehydration, malabsorption, and growth retardation. Treatment with different drugs have been used: Metronidazole (MTZ) (efficiency 73% to 100%), furazolidone, nitazoxanide, and benzimidazoles (albendazole and mebendazole) (26). Mutations in *G. intestinalis* ferredoxin oxidoreductase gene play a role in metronidazole resistance. In Iran, ferredoxin and GINR (*G. lamblia* nitroreductase) genes were investigated in 40 isolates from 38 symptomatic and 2 MTZ-resistant cases; accordingly, nitazoxanide could be used instead of MTZ due to the low mutations in these genes in symptomatic and resistant cases, and the resistance mechanisms were different as well. Therefore, a high ferredoxin mutation was detected in MTZ-resistant cases, and the number of resistant *G. intestinalis* isolates was increased (27).

Amoebiasis

Entamoeba histolytica (*E. histolytica*) is the causative agent of amoebiasis, affecting 500 million people annually. The parasite is transmitted by faecal-oral route and may invade other tissues, mainly liver. MTZ is the most common frequent drug choice for intestinal amoebiasis and amoebic liver abscesses. Although the mode of action is not fully understood, it inhibits DNA synthesis and damage to DNA, proteins, and other cell components by oxidation, as studied from other microorganisms (28). Pathogens develop different resistance mechanisms to MTZ; these mechanisms are associated with altered reduction efficiency, drug inactivation, decreased drug uptake, and increased DNA damage. Clinical MTZ-resistant *E. histolytica* isolates have been identified; however, in vitro resistant isolates have not yet been achieved. MTZ resistance in *E. histolytica* is linked with high iron-containing superoxide dismutase and peroxiredoxin as well as low expression of ferredoxin 1 and flavin reductase (29).

Trichomoniasis

Trichomoniasis is caused by *Trichomonas vaginalis* (*T. vaginalis*) and is the most common non-viral sexually transmitted infection in the world, with 276 million new cases per year. In Turkey, the frequency of *T. vaginalis* in different groups has been reported between 0.3% and 9% in recent studies. The first treatment choice of trichomoniasis is 5-nitroimidazole compounds; among these, MTZ and tinidazole are the most commonly recommended and used drugs. However, MTZ resistance has been reported in various countries since 1962. A study from Aydin presented the MTZ-resistant isolates for the first time in Turkey and reported 7.5% (3 out of 40) in vitro resistance among *T. vaginalis* isolates (30). Tinidazole, ornidazole, furazolidone, and topical pramoxine are currently available drugs used in MTZ-resistant cases. Nitazoxanide, a broad spectrum and low toxicity drug, was found to be effective in MTZ-resistant *T. vaginalis* in vitro and in clinically resistant cases (31). *T. vaginalis* trophozoites use low redox-potent electron-transporting proteins such as pyruvate ferredoxin oxidoreductase (PFOR) and ferredoxin. The reduced PFOR activity of the five-nitroimidazole resistance may be due to the changing structure of the hydrogenosome, the unexpected redox potential in ferredoxin, or intracellular ferredoxin reduction. In the recent studies, genetic markers of MTZ resistance are being investigated. Totally, 72 single nucleotide polymorphisms (SNPs) were related to MTZ resistance in clinical and laboratory isolates of *T. vaginalis*. Some of these identified SNPs were related to resistance (eg., Pfor gene) and drug activation (32).

Nematode infections

Parasitic helminth infections are common in developing countries; *Ascaris lumbricoides* (*A. lumbricoides*) infect 800 million people worldwide. In endemic countries, school children are treated with albendazole or mebendazole twice a year to prevent helminth infections. Benzimidazole (BZ) derivatives (albendazole,

fenbendazole, oxfendazole, mebendazole, and triclabendazole) are widely used to treat nematode diseases, which disrupt tubulin formation (33). Albendazole has been used for the treatment of helminth diseases for about 20 years; however, the presence of resistant isolates has been reported. Molecular tests have been applied in recent years to detect resistance in threadworm species. Different β-tubulin paralogs in the strongyloid and ascarid genomes cause misperception. BZ resistance is common among isotype 1; however, it is rare among isotype 2. In a previous study, no association was found between resistance to changes in the four separate β-tubulin genes in *A. lumbricoides*. The study reported that resistance may not be due to genetically resistant parasites but to other mechanisms such as drug metabolism (34). In whipworm, *Trichuris trichiura*, only one β-tubulin gene was found to be present in a specific isotype. The frequency was found to be increased in the treated cases, which was considered as a candidate for resistance development (35).

Ectoparasites

Pediculus humanus var. capitis (head louse), *P. humanus var. corporis* (body louse), and *Phthirus pubis* (pubic louse) are the louse species that parasitize on humans. These are permanent and obligate ectoparasites that feed with blood and cause pediculosis. The application of topical insecticides is the most effective method in pediculosis treatment. Today, for the treatment of lice, pediculicides such as natural pyrethrins (pyrethrum), synthetic "pyrethroid" (permethrin, phenothrin), organochlorine (indole), organophosphorus (malathion), and carbamate (carbaryl) are commonly used (36). Pyrethrin/pyrethroids and Dichlorodiphenyltrichloroethane target the domain in the voltage-sensitive sodium channel (VSSC) nervous system and increase the sodium flux. They result in neuromuscular paralysis and death by nerve depolarization and hyperexcitations. The widespread use of insecticides and the lack of appropriate replacements cause the development of resistance in pediculosis. One of the resistance mechanisms to pyrethrins or pyrethroids is the target region insensitivity of knockdown resistance (kdr), a heritable feature. Three-point mutations (M815I, T917I, and L920F) in the transmembrane segment were found, and these were present as haplotypes in the permethrin-resistant head lice populations (37). In a study from the United States, 908 bp VSSC α-subunit gene region was studied and the number of resistant lice was increased in comparison with that in the previous years (38).

Scabies is a dermal infection in humans caused by *Sarcoptes scabiei* (*S. scabiei*). It is still an important public health problem in the world, especially in developing countries. Permethrin (5% cream) is used for the first-step treatment in many countries; however, esdepalletrin is used in France instead of permethrin. Other common acaricides are benzyl benzoate 10% to 25%, crotamiton, or oral gum. The intensive use of pyrethroid compounds over the last 30 years has led to the development of resistance mechanisms in many arthropods (39). SNPs play an important role in resistance to pyrethroids in some arthropods. In vitro studies have revealed that the susceptibility of *S. scabiei* to permethrin is gradually reduced by repeated administration. In addition, an SNP at codon 733 in the VSSC gene is related with permethrin resistance in vivo and in vitro studies (40).

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – H.E., E.M.; Design – S.E., H.E.; Supervision – S.E Materials – H.E., E.M.; Data Collection and/or Processing – E.M., H.E.; Analysis and/or Interpretation – S.E., H.E.; Literature Search -H.E., S.E., E.M.; Writing Manuscript – E.M., H.E.; Critical Review – S.E.

Conflict of Interest: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

1. Torgerson PR, Devleesschauwer B, Praet N, Speybroeck N, Willingham AL, et al. World Health Organization estimates of the global and regional disease burden of 11 foodborne parasitic diseases, 2010: a data synthesis. PLoS Med 2015; 12: e1001920. [CrossRef]
2. Andrews KT, Fisher G, Skinner-Adams TS. Drug repurposing and human parasitic protozoan diseases. Int J Parasitol Drugs Drug Resist 2014; 4: 95-111. [CrossRef]
3. WHO. World Health Organization. Fact sheet on the World Malaria Report 2014. Available from: http://www.who.int/malaria/media/world_malaria_report_2014/en
4. Cooper RA, Hartwig CL, Ferdig MT. Pfcrt is more than the Plasmodium falciparum chloroquine resistance gene: a functional and evolutionary perspective. Acta Trop 2005; 94: 170-80. [CrossRef]
5. Okell LC, Griffin JT, Roper C. Mapping sulphadoxine-pyrimethamine-resistant Plasmodium falciparum malaria in infected humans and in parasite populations in Africa. Sci Rep 2017; 7: 7389. [CrossRef]
6. Takala Harrison S, Jacob CG, Arze C, Cummings MP, Silva JC, Dondorp AM, et al. Independent emergence of artemisinin resistance mutations among Plasmodium falciparum in Southeast Asia. J Inf Dis 2015; 211: 670-9. [CrossRef]
7. Wang Z, Wang Y, Cabrera M, Zhang Y, Gupta B, Wu Y, et al. Artemisinin resistance at the China-Myanmar Border and association with mutations in the k13 propeller gene. Antimicrob Agents Chemother 2015; 59: 6952-9. [CrossRef]
8. Li J, Chen J, Xie D, Monte Nguba SM, Eyi JU, Matesa RA, et al. High prevalence of pfmdr1 N86Y and Y184F mutations in Plasmodium falciparum isolates from Bioko Island, Equatorial Guinea. Pathog Global Health 2014; 108: 339-43. [CrossRef]
9. Henry M, Briolant S, Zettor A, Pelleau S, Baragatti M, Baret E, et al. Plasmodium falciparum Na+/H+ exchanger 1 transporter is involved in reduced susceptibility to quinine. Antimicrob Agents Chemother 2009; 53: 1926-30. [CrossRef]
10. Antony HA, Parija SC. Antimalarial drug resistance: an overview. Trop Parasitol 2016; 6: 30-41. [CrossRef]
11. Reed MB, Saliba KJ, Caruana SR, Kirk K, Cowman AF. Pgh1 modulates sensitivity and resistance to multiple antimalarials in Plasmodium falciparum. Nature 2000; 403: 906-9. [CrossRef]
12. Suwanarusk R, Chavchich M, Russell B, Jaidee A, Chalfein F, Barends M, et al. Amplification of pvmdr1 associated with multidrug-resistant Plasmodium vivax. J Infect Dis 2008; 198: 1558-64. [CrossRef]
13. Nyunt MH, Han JH, Wang B, Aye KM, Aye KH, Lee SK, et al. Clinical and molecular surveillance of drug-resistant vivax malaria in Myanmar (2009-2016). Malar J 2017; 16: 117. [CrossRef]
14. Montazeri M, Sharif M, Sarvi S, Mehrzadi S, Ahmadpour E, Daryani A. A systematic review of in vitro and in vivo activities of anti-Toxoplasma drugs and compounds (2006-2016). Front Microbiol 2017; 8: 25. [CrossRef]
15. Silva LA, Reis-Cunha JL, Bartholomeu DC, Vítor RW. Genetic polymorphisms and phenotypic profiles of sulfadiazine-resistant and

sensitive Toxoplasma gondii isolates obtained from newborns with congenital toxoplasmosis in Minas Gerais, Brazil. PloS One 2017; 12: e0170689. [CrossRef]
16. Meneceur P, Bouldouyre MA, Aubert D, Villena I, Menotti J, Sauvage V, et al. In vitro susceptibility of various genotypic strains of Toxoplasma gondii to pyrimethamine, sulfadiazine, and atovaquone. Antimicrob Agents Chemother 2008; 52: 1269-77. [CrossRef]
17. Baraka V, Ishengoma DS, Fransis F, Minja DTR, Madebe RA, Ngatunga D, et al. High-level Plasmodium falciparum sulfadoxine-pyrimethamine resistance with the concomitant occurrence of septuple haplotype in Tanzania. Malar J 2015; 14: 439. [CrossRef]
18. Aspinall TV, Joynson DH, Guy E, Hyde JE, Sims PF. The molecular basis of sulfonamide resistance in Toxoplasma gondii and implications for the clinical management of toxoplasmosis. J Infect Dis 2002; 185: 1637-43. [CrossRef]
19. Jeffers V, Kamau ET, Srinivasan AR, Harper J, Sankaran P, Post SE, et al. TgPRELID, a mitochondrial protein linked to multidrug resistance in the parasite Toxoplasma gondii. mSphere 2017; 2: e00229. [CrossRef]
20. Leta S, Dao TH, Mesele F, Alemayehu G. Visceral leishmaniasis in Ethiopia: an evolving disease. PLoS Neg Trop Dis 2014; 8: e3131. [CrossRef]
21. Radmanesh M, Omidian E. The pulsed dye laser is more effective and rapidly acting than intralesional meglumine antimoniate therapy for cutaneous leishmaniasis. J Dermatolog Treat 2017; 28: 422-5. [CrossRef]
22. Kazemi-Rad E, Mohebali M, Khadem-Erfan MB, Hajjaran H, Hadighi R, Khamesipour A, et al. Overexpression of ubiquitin and amino acid permease genes in association with antimony resistance in Leishmania tropica field isolates. Korean J Parasitol 2013; 51: 413-9. [CrossRef]
23. Kazemi-Rad E, Mohebali M, Khadem-Erfan MB, Saffari M, Raoofian R, Hajjaran H, et al. Identification of antimony resistance markers in Leishmania tropica field isolates through a cDNA-AFLP approach. Exp Parasitol 2013; 135: 344-9. [CrossRef]
24. Barrera MC, Rojas LJ, Weiss A, Fernandez O, McMahon Pratt D, Saravia NG, et al. Profiling gene expression of antimony response genes in Leishmania (Viannia) panamensis and infected macrophages and its relationship with drug susceptibility. Acta Trop 2017; 176: 355-63. [CrossRef]
25. Lane S, Lloyd D. Current trends in research into the waterborne parasite Giardia. Crit Rev Microbiol 2002; 28: 123-47. [CrossRef]
26. Gardner TB, Hill DR. Treatment of giardiasis. Clin Microbiol Rev 2001; 14: 114-28. [CrossRef]
27. Galeh TM, Kazemi A, Mahami-Oskouei M, Baradaran B, Spotin A, Sarafraz S, et al. Introducing nitazoxanide as a promising alternative treatment for symptomatic to metronidazole-resistant giardiasis in clinical isolates. Asian Pac J Trop Med 2016; 9: 887-92. [CrossRef]
28. Löfmark S, Edlund C, Nord CE. Metronidazole is still the drug of choice for treatment of anaerobic infections. Clin Infect Dis 2010; 50(Suppl): S16-23. [CrossRef]
29. Wassmann C, Hellberg A, Tannich E, Bruchhaus I. Metronidazole resistance in the protozoan parasite Entamoeba histolytica is associated with increased expression of iron-containing superoxide dismutase and peroxiredoxin and decreased expression of ferredoxin 1 and flavin reductase. J Biol Chem 1999; 274: 26051-6. [CrossRef]
30. Ertabaklar H, Yaman Karadam S, Malatyalı E, Ertuğ S. Investigation of in vitro metronidazole resistance in the clinical isolates of Trichomonas vaginalis. Mikrobiyol Bul 2016; 50: 552-8. [CrossRef]
31. Bouchemal K, Bories C, Loiseau PM. Strategies for prevention and treatment of Trichomonas vaginalis infections. Clin Microbiol Rew 2017; 30: 811-25. [CrossRef]
32. Bradic M, Warring SD, Tooley GE, Scheid P, Secor WE, Land KM, et al. Genetic indicators of drug resistance in the highly repetitive genome of Trichomonas vaginalis. Genome Biol Evol 2017; 9: 1658-72. [CrossRef]
33. Massara CL, Enk MJ. Treatment options in the management of Ascaris lumbricoides. Expert Opin Pharmacother 2004; 5: 529-39. [CrossRef]
34. Krücken J, Fraundorfer K, Mugisha JC, Ramünke S, Sifft KC, Geus D, et al. Reduced efficacy of albendazole against Ascaris lumbricoides in Rwandan schoolchildren. Int J Parasitol Drugs Drug Resist 2017; 7: 262-71. [CrossRef]
35. Rashwan N, Scott M, Prichard R. Rapid genotyping of β-tubulin polymorphisms in Trichuris trichiura and Ascaris lumbricoides. PLoS Negl Trop Dis 2017; 11: e0005205. [CrossRef]
36. Durand R, Bouvresse S, Berdjane Z, Izri A, Chosidow O, Clark JM. Insecticide resistance in head lice: clinical, parasitological and genetic aspects. Clin Microbiol Infect 2012; 18: 338-44. [CrossRef]
37. Clark JM, Yoon KS, Kim JH, Lee SH, Pittendrigh BR. Utilization of the human louse genome to study insecticide resistance and innate immune response. Pest Biochem Physiol 2015; 120: 125-32. [CrossRef]
38. Gellatly KJ, Krim S, Palenchar DJ, Shepherd K, Yoon KS, Rhodes CJ, et al. Expansion of the knockdown resistance frequency map for human head lice (Phthiraptera: Pediculidae) in the United States using quantitative sequencing. J Med Entomol 2016; 53: 653-9. [CrossRef]
39. Thomas J, Peterson GM, Walton SF, Carson CF, Naunton M, Baby KE. Scabies: an ancient global disease with a need for new therapies. BMC Infect Dis 2015; 15: 250. [CrossRef]
40. Andriantsoanirina V, Izri A, Botterel F, Foulet F, Chosidow O, Durand R. Molecular survey of knockdown resistance to pyrethroids in human scabies mites. Clin Microbiol Infect 2014; 20: 139-4. [CrossRef]