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Review article

Drug resistance in parasites: A review of mechanisms, drivers, and mitigation strategies

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ABSTRACT

Background: Drug resistance among parasitic pathogens is a pressing public health concern. This review highlights the emergence of resistance among parasites, its impacts, key drivers and potential solutions. The objective of this review is to provide an overview of the harms and risks associated with drug resistance among parasites of public and veterinary health concerns. Resistance is often attributed to mutations in target proteins, altering their binding and efficacy. Other factors contributing to resistance include increased drug exposure due to self-medication, genetic diversity of parasites, and environmental factors like climate change. The emergence of these resistance-associated mutations may lead to catastrophic consequences, like inefficient drug-based control and elimination programs, rebounds in cases, and the widespread occurrence of severe pathological consequences due to unchallenged infections. Strategies to address drug resistance include surveillance, innovative population-based drug administration methodologies, vaccine development, and non-pharmaceutical interventions (i.e., improved sanitation and hygiene, and community health education). Addressing drug resistance requires a multi-faceted approach to prevent its further spread and ensure effective parasite control and elimination.

Introduction

Several parasitic pathogens have been reported to have reduced efficacy against frontline clinical and preventative treatments. Among protozoans, drug resistant *Plasmodium* and *Giardia* remain of public health concern. Drugs used in clinical and public health interventions against *Plasmodium* species *(*spp.), like sulfadoxine and pyrimethamine, chloroquine and artemisinin derivatives, have been reported to have reduced efficacy [1]. Resistance against these drugs among malarial parasites are through mutations in target proteins, overexpression of efflux pumps, and mutation-induced changes in catalytic activity in certain enzymes [1]. In refractory *Giardia* spp. infections, resistance is found against metronidazole and benzimidazoles [2]. Resistance against metronidazole and other nitroheterocycle drugs are due to reduced expression of enzymes that are involved in drug activation or overexpression of efflux pumps [2]. Meanwhile, resistance to benzimidazoles is conferred by mutations in the β-tubulin gene and being able to resist the reactive oxygen species assault caused by the drug [2].

Among helminths, drug resistance in soil-transmitted helminths (STH), schistosomes and filarial nematodes are of particular concern. In STH like *Ascaris lumbricoides, Trichuris trichiura* and hookworms, drug resistance against benzimidazole drugs that are used in Mass Drug Administration (MDA) as control and elimination strategy has been of rising concern recently [3]. Resistance conferring

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mutations within the β-tubulin gene has been reported in STH in South and Central America and Africa [4, 5]. Praziquantel resistance in *Schistosoma mansoni (S. mansoni*) has been documented in Egypt, Kenya, and Senegal [6]. Praziquantel disrupts calcium signalling through agonizing voltage-gated calcium channels in helminths. The influx of calcium result muscle contraction and surface modifications that allow for easier immune clearance of the parasite [7]. Alterations in 20 of the 23 amino acid residues lining the binding site for praziquantel in *Schistosoma mansoni* caused reduced sensitivity to the drug [7]. The target protein in *S. mansoni* is *S. mansoni* transient receptor potential melastatin PZQ channel ($SmTRPM_{PZQ}$). The N1388T mutation in the $SmTRPM_{PZQ}$ protein caused a loss of PZQ-evoked activity [8].

A growing concern regarding drug resistance in parasites that is often overlooked is the emergence of resistant arthropod vectors of parasitic protozoans or helminths. Pyrethroid resistance in *Anopheles* spp. is linked with metabolic detoxification through over expression of several enzymes, like cytochrome p450s, esterases and glutathione S-transferases [9]. Genetic markers for insecticide resistance has been found in anopheline mosquitoes from Tanzania, Mozambique, Malawi and Democratic Republic of Congo [10]. Resistance in these vectors may complicate the control and elimination of vector-borne diseases. Moreover, resistance in vectors also add to the complications of treatment-resistant malaria.

The objective of this narrative review is to provide an overview regarding the harms and risks associated with drug resistance among parasites of public and veterinary health concern. Highlighted herein are drug resistance against anthelmintics that are due to mutations in key protein-encoding genes. The impact of these mutations, key drivers of their emergence and the strategies to subvert the threat of resistance among parasites are also discussed herein.

Drug resistance against anthelmintics

Benzimidazole

For benzimidazoles, single nucleotide polymorphisms (SNPs) affect the gene responsible for producing the β-tubulin protein, a crucial component of the parasite's cytoskeleton [11]. These mutations disrupt the protein's structure, preventing the efficient binding of benzimidazole drugs and rendering the parasite resistant to their effects. Seven of such mutations that cause amino acid substitutions have been reported among hookworms: Q134H, F167Y, E198A, E198K, E198V, F200Y, and F200L [5, 12–17]. Aside from hookworms, these mutations have been reported in *Ascaris lumbricoides* and *Trichuris trichiura* [4].

Macrocyclic lactones

Resistance to macrocyclic lactones, like ivermectin, is caused by mutations in ligand-gated chloride channels of the helminth's nervous system. Binding of the macrocyclic lactones to these channels cause influx of chloride ions resulting in paralysis and subsequently death [18]. In subunit proteins (e.g., glc-5, lgc-37, and avr-14) composing these glutamate-gated channels, mutations like A159V, K159R, and L256F are linked with resistance among *Haemonchus conturtus* and *Dirofiliaria immitis* [19]. These mutations negatively affect the binding of the drug ligand to the binding site leading to decreased sensitivity to chloride ions. The influx of chloride ions on the effector cell results in hyperpolarization leading to parasite killing does not occur.

Praziquantel

Another drug that is threatened by mutation-associated resistance is praziquantel. Although resistance has yet to be fully confirmed among parasites susceptible to praziquantel, reduced efficacy after several rounds of treatment have been reported among schistosomes [18]. Like ivermectin where parasite death is achieved through disruption of nervous activity, praziquantel disrupts calcium signalling through agonizing voltage-gated calcium channels in helminths. The influx of calcium result muscle contraction and surface modifications that allow for easier immune clearance of the parasite [7]. Alterations in 20 of the 23 amino acid residues lining the binding site for praziquantel in *Schistosoma mansoni* caused reduced sensitivity to the drug [7]. The target protein in *S. mansoni* is *S. mansoni* transient receptor potential melastatin PZQ channel ($SmTRPM_{PZQ}$). The N1388T mutation in the $SmTRPM_{PZQ}$ protein caused a loss of PZQ-evoked activity [8]. Moreover, genome wide analysis of isolates with laboratory induced praziquantel resistance showed higher polymorphisms in genes coding for the $SmTRPM_{PZQ}$ protein. In addition to this, however, increased variability was also seen in ATP-binding cassette transporter genes, which are members of p-glycoprotein family that pump-out drugs from the parasite's cells [20, 21].

Levamisole and monepantel

Other drugs, like levamisole and monepantel, has also been reported to be negatively affected by mutations in their target proteins. Both of these drugs work as agonists of nicotinic acetylcholine receptors, which result in uncontrolled influx of ions into muscles cells of the worm culminating in paralysis then death [5]. Thus, mutations in this receptor enable the parasite to potentially resist treatment. Genome level studies in multidrug resistant *Haemonchus contortus* reveal that S168T mutation in the *arc-8* gene result in decreased drug efficacy due to changes in the molecular interactions within the binding pocket of the ARC-8 protein [22]. Monepantel resistance is similarly invoked by mutations in the target receptors but a deletion in the gene of coding a target protein has also been found to correlate with resistance [23].

Impact of resistance-associated mutations

Mutations associated with anthelmintic resistance undermine the efficacy of dewormers through altering the target proteins, in almost all cases. These alterations negatively affect the binding of the drug ligands with their target receptors thereby resulting in the suboptimal activation of the intended mechanism of parasite killing. This is exemplified by mutations in the β-tubulin gene that is implicated in the resistance against benzimidazole drugs [11]. In other cases, mutations cause alterations not in the structure of the protein target but rather in regulatory enzymes or genes that increase or decrease the expression of efflux pumps. In this situation, the drug may still be able to bind with the target protein, but the mutations have caused over expression of pumps that are able to reduce the drug concentration within the vicinity of the target receptor hindering its action. In praziquantel resistance, over expression of ABC transporters have been linked to mutations that disrupt the regulatory mechanism involved in efforts to eject and resist drug molecules [7]. An alternate method of resistance has been to lose the target protein altogether through gene deletions or reduced expression. This is particularly true in levamisole resistance where target receptor isotypes may either have significant indel mutations or have altered gene expression [18, 22]. The rise of these resistant helminths will undermine control and elimination efforts which could lead to negative effects on human and animal health.

There are several implications brought about by the rise of drug resistance in parasitic pathogens. First, the development of resistance in parasites presents a threat against the current line of treatments. A trend that is used to temporarily combat this threat is to use combination drugs, similar to what is done in public health interventions against filarial nematodes that utilize ivermectin, diethylcarbamazine, and albendazole [24]. The rationale for this combination treatment is to attack different stages of the lifecycle, increase effectiveness and hasten the elimination. The same scenario is done in the veterinary field. However, a negative consequence that was observed as a result of combination drug treatments is the emergence of multidrug resistant helminths, like hookworms [25].

Second, the emergence of resistant parasitic pathogens may result in adverse effects regarding their control and elimination. Most parasitic infections of public health concern are controlled by pharmacological interventions; hence the emergence of resistance is an issue that can undermine interventions that are currently implemented. For instance, in the Philippines, MDA-based programs against STH, schistosomiasis and lymphatic filariasis have resulted in relative successes in reducing the prevalence and burden of these parasitic infections [26]. However, the protracted implementation of these control required for it to be effective adds positive selective pressure to the worms for them to acquire resistance mechanisms [5]. And the emergence of these resistant helminths could lead to rebounds in prevalence and the pathological consequences these infections bring about.

Key drivers of drug resistance among parasites

There are several biological factors that can contribute to the development of drug resistance in parasites. First, genetic variations that arise naturally may inadvertently result in drug resistance. This has been hypothesized and observed in drug resistant *Plasmodium* spp. Genetic mutations that confer resistance, whether naturally occurring or induced by the environment, is influenced by evolutionary genetic structure and dynamics, and infection-related factors like prevalence and transmission rates [27]. In *Plasmodium*, genetic and geographic diversification through wide dissemination of the vector-borne parasite across the continental Southeast Asia is one of the key determinants, together with drug administration selective pressure,

spelled the emergence of artemisinin resistance [28]. Among nematodes, genetic diversity, as studied in several nuclear and mitochondrial genes, of *H. contortus* has been linked to the emergence of multi-drug resistance because of its increased polymorphism rates, enlarged population effective size and increased migration rates through within country and international trade of their goat and sheep hosts [29, 30]. When this propensity for genetic diversification is met with selective pressure caused by repeated drug exposure, resistance may arise in a hastened pace [31].

There are several environmental factors that may contribute to the emergence and spread of drug resistance. Protracted drug exposure through repeated administration (i.e., MDA in humans and routine deworming in animals) exerts positive selective pressure to the parasites to mutate drug targets in order to evade the mechanism of action of the drug [11]. Repeated exposure selects for populations that contain these resistance mutations until a large percentage of resistant parasites remains and resistance emergence becomes apparent. A recent modeling study the occurrence resistance-associated mutations against benzimidazoles may occur within 10 years of continued deworming using the same type of drugs within school-aged children [32]. It is important to remember, however, that resistance-associated mutations against benzimidazoles are counteracted by fitness costs: some mutations confer resistance but may not be suited for survival in the field [33].

Aside from the untoward effects exerted by pharmacological interventions, climate change may also influence the emergence of resistant parasites. Indirectly, the increase in global temperatures may increase parasite transmissions and widen the spread of parasites and their vectors leading to increased transmission intensity within a wider geographic range thereby affecting the emergence and spread of resistant parasites, similar to what is theoretically proven in *Plasmodium falciparum* [34]. Similarly, a recent modelling study of cyathostomins from horses using projections from New Zealand showed that climate change may increase the survival rate of these nematodes [35]. Favored parasite survival leads to population increase; this accelerates resistance when combined with pharmacological interventions [35]. These results show that drivers for the emergence of drug resistance may be attributed to the inherent capacity of the parasite for diversification, the positive selective pressure induced by drug administration and the potential consequences of climate temperatures rising due to climate change and global warming.

The impact of human behavior

Factors that influence the emergence of drug resistance are not limited to biological and environmental factors but also may include behavioral factors related to the susceptible hosts. Speaking of drug resistance in the helminths of public health concern, participation in and adherence to MDA programs may affect the occurrence of drug-resistant parasites. In areas where MDA has been practiced for long periods of time, repeated deworming may lead to poor compliance due to MDA fatigue. This phenomenon has been reported in the Philippines in endemic areas for schistosomiasis that have received protracted praziquantel MDA [36]. Aversion to participate in these deworming programs may lead to underdosing, inconsistent treatment coverage and incomplete parasite killing which could result in drug resistance in the target parasites. Persistence of infections through noncompliance or undercompliance to the MDA program enables parasite to circulate within populations where they may encounter sublethal drug doses that can pressure them to develop resistance-associated mutations [37].

Aside from poor treatment coverage and compliance, self-treatment using commercially available drugs may also fast-track the occurrence of resistant parasites. This is of particular concern in veterinary medicine where farmers may obtain anthelmintic drugs and self-treat their animals. Misuse of dewormers and other antimicrobials by farmers on their animals may be brought about by poor knowledge leading to indiscriminate use and improper dosing, lack of access to proper veterinary care, and cost considerations [38]. However, self-treatment together with poor compliance to the proper treatment course has also been outlined as a contributing factor to the emergence of malaria resistance [39]. **Douine *et al.*** [37], in their study in the French Guyana, found that more than 50% of their respondents were self-treating against malaria by using artemisinin derivatives and a number of these has not been diagnosed prior to commencement of treatment. Poor adherence to proven treatment protocols may result in drugs misuse and intake of sublethal doses that promote

the occurrence of drug resistance when done repeatedly [28].

Strategies to prevent and slowdown the emergence of resistance

The emergence and potential spread of drug-resistant parasites should be met with appropriate mitigation strategies. First, appropriate surveillance on the occurrence of resistant parasites should be done according to the recommendations of the World Health Organization. For STH and schistosomiasis, it has been posited that evaluation of efficacy of the drugs used in MDA should be done every four to five years regardless of resistance suspicion [40]. In addition to efficacy evaluation using egg reduction tests, molecular assessments on mutations associated with drug resistance should also be conducted. For instance, **George *et al.*** [39] used deep amplicon sequencing to assess the occurrence of benzimidazole resistance-associated mutations in hookworms before and after a round albendazole treatment in Kpandai District, Ghana. Integration of microscopy-based epidemiological investigations with molecular analysis related to resistance mechanism assessment will provide a better understanding of drug resistance occurrence and development.

Second, innovative ways of curative and preventative drug administration for at risk populations should be considered. Targeted treatment of diagnosed individuals can be a viable alternative to MDA. This has been utilized in for the control and elimination of *Opisthorchis viverrini* in Northeastern Thailand via the Lawa Model program [42]. The advantage of having a diagnostic part prior to drug administration is that respondents may be more likely to comply with treatment if they know that they are infected. Moreover, it limits the selective pressure added to parasite populations by targeting the portion that is infecting humans leaving those in refugia less pressured to acquire resistance-associated mutations [43]. In veterinary medicine, targeted treatment has been raised as an alternative to indiscriminate deworming of herds and flocks; treatment is only provided to segments of the population with severe infections only [43]. While targeted treatment presents a viable alternative to mass administration of anthelmintics, it should be noted that this strategy may not be suitable for the control of parasitic diseases with high prevalence within a wide range of geographical locations, diseases with acute and severe pathogenesis, and parasites that have modes of infections that are suitable with certain human demographics (e.g., people in poverty infected with STH).

Vaccine development against helminths present hope in the strife against resistant parasites. Human vaccine against hookworms have been explored and has received considerable successes. Recombinant vaccines containing *Necator americanus* glutathione-S-transferase and aspartic protease have been developed [44]. Both vaccine targets are enzymes that enable hookworms to feed on blood. These vaccines are currently within phase 1 to phase 2 vaccine trial [45]. A recent trial that utilized an aspartic protease recombinant target with Alhydrogel adjuvant reported satisfactory immune response amongst respondents (e.g., high levels of IgG induced) with minimal adverse effects [46]. Vaccine development for parasites of public and veterinary health concern provide an alternative method of preventing infections without the use of pharmacological interventions thereby limiting the emergence and spread of drug resistance.

Another method of infection control and elimination that may lessen the probability of resistance emergence among parasites is the implementation of non-pharmaceutical interventions. Improvement of water, sanitation, and hygiene (WASH) in areas where neglected tropical diseases are prevalent has been advocated to reduce the occurrence of infections [47]. Improving hygienic practices have been linked to reductions in odds of infections in STH: 38–46% reduced odds of *A. lumbricoides*, 39–42% reduced odds of *T. trichiura* and 40% reduced odds of hookworm infections [48]. Aside from improving WASH infrastructures, community health education and efforts to let the community participate in control and eliminate parasitic infections should be done. Addressing health issues, like anthelmintic resistance, through One Health may offer alternative interventions that can alleviate parasitic resistance while limiting the emergence of refractory efficacy of drug administration.

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Competing interest

The author has no conflict of interest to declare.

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