Resistance testing in parasites: a review

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Introduction

The burden of parasitic diseases in the world is enormous and 41% of the world's population lives in areas where malaria is transmitted. Each year 350–500 million cases of malaria occur worldwide, and over one million people die, most of them young children in sub-Saharan Africa [1]. About 2 billion people are affected worldwide, of whom 300 million suffer associated severe morbidity [2]. At least 1 billion people – one sixth of the world's population, or 1 in 6 persons, suffer from one or more neglected tropical diseases (NTDs), most of which are parasitic [3].

Some parasitic diseases are endemic in Australia with potential of outbreaks in schools (head lice), daycare centres (*Giardia*), swimming pools (*Cryptosporidium*) and amongst Indigenous Australians in the 'top end' of the country [4].

Failure to respond to antiparasitic drugs is a major concern in many parts of the world. The biggest concern is with antimalarial drugs for *Plasmodium falciparum*, which has now become resistant to a number of drugs. Resistance has also been reported in other protozoa (*Leishmania*), helminths (particularly veterinary helminths) and in arthropods (lice and mites). **Testing for resistance in parasites is very difficult, not well standardised and hence, not routinely available in diagnostic laboratories.** This article will briefly review the methods that have been used by various scientists and highlight the complexity of testing in parasites. Some of the techniques are developed for testing effects of new antiparasitic drugs and can be used to look for resistance.

Failure to respond is not always resistance

Failure to respond to treatment is not always due to resistance to the drug (see table 1). True resistance may be inherent or acquired. Most antiparasitic drugs have a specific and narrow spectrum of activity. Moreover, the complex life cycle of parasites means passing through different stages from eggs or cysts to trophozoites or larvae and adult forms. Each phase is morphologically very different to the previous stage, particularly in helminths and drugs that act on one stage may be completely inactive against other stages. Acquired resistance is due to misuse or improper use of the drug (e.g. chloroquine in malaria or antimony compounds in *Leishmania* and antihelminthic drugs in veterinary parasites)

Mechanisms of resistance

The mechanisms of resistance in parasites have been studied and essentially are similar to other microorganisms; namely efflux, modulation of transport, target modification, bypass, transport defects, drug modification, alteration in binding affinities, reduced concentration or lack of enzyme, enzyme turnover etc. Genetic markers for these have been identified and can be detected by molecular techniques [5,6]. However these are not available routinely.

Why is it so difficult to test for resistance?

Parasites are difficult to isolate in simple culture methods, such as artificial media. It is possible to culture some protozoa, e.g. *Acanthamoeba* on non-nutrient agar, but others are not. Many parasites have a complex life cycle involving different hosts and different stages will require different conditions of growth. Cell lines and experimental animals are difficult to maintain in routine laboratories and procedures to isolate and maintain parasites in these can be very labour intensive and costly.

Table 1: Reasons for failure to respond to treatment.

Reinfection
Inadequate drug levels Wrong dose Improper uptake (diet, gut factors) Interaction (drugs, alcohol)
Immuno-suppression
Sequestration in tissues (pancreas, gall bladder)
True resistance – inherent or acquired
Unknown causes

Standardisation of the method used, especially culture methods is of growing importance. Two networks have been created with the objective of using a standardised in vitro assay method: the Paludisme network of laboratories in French-speaking countries and the Red Amazonica para la Vigilancia de la Resistencia a las Drogas Antimalaricas. Since 2000, the World Health Organization (WHO) has been working with the University Sains Malaysia in Penang, Malaysia, which manufactures pre-dosed plates for *in vitro* tests. Quality control of the plates has been supervised since 2002 by the Institut de Medecine Tropicale du Service de Sante des Armees in Marseille, France [7].

How is resistance detected?

Resistance may be either presumed (clinical failure) or tested for in the laboratory. Various ways are:

- Case reports and passive detection of treatment failure.
- *In vitro* tests:
  - macro- and micro-techniques (microscopy, EIA etc).
  - faecal egg count reduction test, comparison with reference strains.
- *In vivo* tests:
  - Animal models, egg hatch test, larval paralysis test, etc.
- Molecular techniques for resistance genes, e.g. *dhfr* gene, *pfmdr1* gene, etc.

Principles of the tests

Table 2 provides a summary of parasites where resistance has been reported and lists the methods used for detection of this resistance. (A full list of references is available on request).

Table 2: Summary of parasites in which resistance is reported, and the methods used.

Parasite (group)	Antiparasitic drugs	Method for resistance testing used
Plasmodium falciparum	all antimalarial	Various
Plasmodium vivax	antifolate	Molecular (*dhfr* mutation)
Acanthamoeba	various	Microtitre assay Tube dilution assay
Entamoeba	metronidazole	Microtitre assay Tube dilutions assay
Giardia	metronidazole	Microtitre assay Animal model
Trichomonas	metronidazole	Microtitre assay Pouch technique
Toxoplasma	pyrimethamine	Molecular (*dhfr* mutation)
Leishmania	antimony compounds, amphotericin B	Various
Trypanosoma brucii	pentamidine, suramin, melarsoprol	Direct counting Enzyme hydrolysis Molecular
Schistosoma	praziquantel	Animal model
Hookworms	pyrantel, mebendazole	Clinical and parasitological criteria
Filarial worms	ivermectin, DEC	Clinical and parasitological criteria
Veterinary helminths	various	Various
Pediculus	permethrin, pyrethrine	Exposing lice *in vitro*
Sarcoptes	permethrin, ivermectin	Exposing mites *in vitro*

Protozoa

In general, it is easier to test for resistance in protozoa [8]. It is possible to culture some as they have a relatively simple life cycle. Resistance in certain protozoa, such as malarial parasite, is very common in certain parts of the world. Resistance in other protozoa, e.g. *Giardia*, is relatively uncommon, although recently this seems to be increasing.

Malaria

Most work has been done on malarial parasites [9] especially *Plasmodium falciparum.* A number of tests including the WHO microtest, radioisotope assay, pLDH (parasite lactate dehydrogenase) enzyme assay, DELI (double site enzyme linked LDH immuno-detection test) and HRP2 (histidine rich protein 2) assay have been developed. Some of these have been approved for use in the field by WHO [10].

Helminths

Helminths are much more difficult to culture. In addition, they pass through a complex life cycle and it is not always possible to grow and test for all stages of the parasite outside its natural host.

Reports of drug resistance have been made in every livestock host and to every anthelmintic class. In some regions of the world, the extremely high prevalence of multi-drug resistance (MDR) in nematodes of sheep and goats threatens the viability of small ruminant industries. Resistance in nematodes of horses and cattle has not yet reached the levels seen in small ruminants, but evidence suggests that the problems of resistance, including MDR worms, are also increasing in these hosts. There is an urgent need to develop both novel non-chemical approaches for parasite control and molecular assays capable of detecting resistant worms. The role of molecular diagnosis for resistance to antiparasitic drugs will be the way of the future. A number of tests have been applied in veterinary parasitology [11] and can be applied to humans, but none have been developed, adapted or adequately validated in human cases.

Arthropods

In general these are tested by exposing the insects to the agent used for treatment in the laboratory and comparing the outcome with maintained colonies of reference strains (fully sensitive) or by combining with results of clinical outcome [12].

Conclusions

Parasitic diseases, particularly malaria, veterinary helminths and soil transmitted helminths, are big global problems. Failure to respond (or resistance to drugs) is increasing. Standardised resistance testing for antiparasitic drugs is currently not available in medical laboratories. Hence, the incidence of resistance in various parasites is not accurately known. The WHO is helping to make some progress in producing standardised field kits for malaria. Given the technical difficulties and complexity of testing parasites and with the advent and progress in molecular biology, these techniques should be available in the future for resistance testing in parasites.

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