Trends in Parasitology

CellPress

Opinion

Integrating metabolomics into the diagnosis and investigation of anthelmintic resistance

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Anthelmintic resistance (AR) in parasitic nematodes poses a global health problem in livestock and domestic animals and is an emerging problem in humans. Consequently, we must understand the mechanisms of AR, including target-site resistance (TSR), in which mutations affect drug binding, and non-target site resistance (NTSR), which involves alterations in drug metabolism and detoxification processes. Because much of the focus has been on TSR, NTSR has received less attention. Here, we describe how metabolomics approaches using *Caenorhabditis elegans* offer the ability to disentangle nematode drug metabolism, identify metabolic changes associated with resistance, uncover novel biomarkers, and enhance diagnostic methods.

Integrating metabolomics into the diagnosis and investigation of anthelmintic resistance

The widespread use of anthelmintics to control parasitic nematode infections in domesticated animals and livestock has led to the rapid evolution of drug resistance across parasite species and threatens the success of parasite control efforts of species that infect humans. As anthelmintic resistance (AR) rises, resistance alleles spread throughout populations, making it more difficult to control parasitic nematode populations [1,2]. Therefore, we urgently need to identify the mechanisms of resistance (MoR) to anthelmintics, establish efficient ways to measure anthelmintic efficacy, improve diagnostic testing, and develop approaches to optimize drug potency.

Resistance mechanisms can be broadly classified into two categories: **target site resistance** (**TSR**) (see Glossary) and **non-target site resistance** (**NTSR**). TSR occurs when mutations alter the protein and prevent the drug from binding effectively. NTSR refers to mutations that affect drug absorption, translocation, sequestration, and elimination. Historically, TSR mechanisms have been the focus in parasitology communities [3–5]. However, NTSR mechanisms are increasingly recognized as putative contributors to resistance [6–9]. In this context, drug-metabolizing enzymes reduce anthelmintic efficacy and thereby contribute to the development of AR. Although the modes of action and TSR for anthelmintic drugs are often the focus of resistance research, evidence and appreciation that NTSR (e.g., altered metabolism) affects drug efficacy is growing [6–11].

Metabolomics offers a promising avenue for advancing our understanding of AR by providing insights into the metabolic processes that affect drug efficacy and resistance mechanisms [12]. Using metabolomics tools, we can uncover how drugs are metabolized and how AR evolves [13]. Metabolomics approaches allow for the identification of novel biomarkers associated with resistance and can inform the development of more effective diagnostic tools and treatment strategies [14–16]. In addition, metabolomics can help to elucidate the interactions between drugs and metabolic pathways, providing a more comprehensive understanding of how resistance mechanisms influence anthelmintic efficacy [17]. By integrating metabolomics with genetic and biochemical data, researchers can develop more targeted and effective strategies to combat AR.

Highlights

The rise of anthelmintic resistance (AR) in nematodes demands urgent identification of resistance mechanisms, better diagnostic tools, and drug optimization strategies.

Although target-site resistance (TSR) has been the traditional focus to understand AR, non-target site resistance (NTSR) is also a significant contributor.

Metabolomics offers powerful approaches to uncover mechanisms of resistance (MoR) by identifying key metabolic pathways, enzymes, and small molecules associated with drug metabolism and AR.

Caenorhabditis elegans is an ideal model organism for studying AR and can define the roles that both TSR and NTSR play across diverse nematode species.

Identifying xenobiotic-metabolizing enzymes (XMEs) could broaden treatment strategies by the discovery of synergistic drug combinations involving enzyme inhibitors, which could lead to more effective anthelmintic treatments.

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Here, we highlight the value of using the free-living nematode *Caenorhabditis elegans* and metabolomics as tools to bridge the knowledge gap of how TSR and NTSR contribute to AR. We focus on how using *C. elegans* and metabolomics can reveal novel strategies to improve drug efficacy, aid in the identification of infection and AR biomarkers, and refine diagnostic testing (Figure 1). Finally, we outline the practical implications of how to translate this work to restore anthelmintic efficacy and slow resistance in parasitic nematode species.

Approaching both sides of the coin to tackle AR: TSR and NTSR

To tackle the growing challenges associated with AR, it is essential to consider the roles that TSR and NTSR contribute to AR, because each involves distinct mechanisms that impact treatment efficacy. TSR occurs when mutations alter a drug's target, reducing anthelmintic efficacy [18–20] (Figure 2A). However, TSR does not explain the full range of resistance to any anthelmintic, which suggests that other non-target site mechanisms must be involved [8,19].

NTSR mechanisms do not directly affect the drug's target but instead modify how drugs are metabolized, distributed, and eliminated within an organism [21–24] (Figure 2B). Therefore, the effect of pharmacotherapy can be decreased by the enhanced inactivation and/or elimination of an active drug by the increased expression and activity of **xenobiotic-metabolizing enzymes** (**XMEs**) [24,25]. By upregulating detoxification pathways or altering drug uptake, nematodes can effectively neutralize the drug before it reaches its intended target [26,27]. To address the role both TSR and NTSR play in AR, we must systematically examine nematode drug metabolism pathways as we create perturbations in TSR to understand MoR comprehensively.

Using *C. elegans* as a model organism to explore TSR mutations and the roles of XMEs in NTSR will allow us to detect key metabolic alterations (e.g., fatty acid metabolism, altered enzymatic activity, increased expression of efflux pumps) and test potential inhibitors of these pathways. These findings can then be validated in parasitic nematodes, where conserved resistance mechanisms

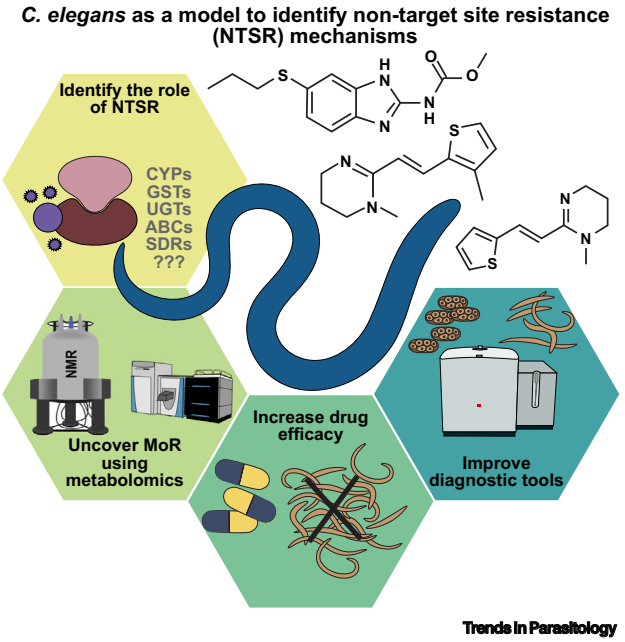

Figure 1. Leveraging metabolomics and *Caenorhabditis elegans* to enhance our understanding of anthelmintic resistance (AR) and advance strategies for treatment and diagnosis. Metabolomics approaches hold great potential to deepen our understanding of anthelmintic mechanisms of resistance (MoR), including non-target site resistance (NTSR), provide strategies to increase drug efficacy to slow resistance, and improve diagnostic tools.

Glossary

Albendazole (ABZ): a broad-spectrum anthelmintic in the benzimidazole drug class used to treat various parasitic nematode infections by inhibiting the formation of microtubules in nematodes and other parasites.
Benzimidazoles (BZs): a class of anthelmintic drugs that inhibit the polymerization of tubulin, thereby disrupting microtubule formation and leading to the death of parasitic nematodes.
(Detoxifying) enzyme inhibitors: chemical compounds that inhibit the activity of enzymes responsible for metabolizing and detoxifying drugs and toxins, potentially enhancing the effectiveness of these substances by preventing their breakdown.
Excretory/secretory products (ESPs): molecules released by helminths into their host environment either through the excretion of 'waste' products or by an active secretory process for 'functional' molecules.
Ivermectin: an anthelmintic that acts as a positive allosteric modulator which selectively opens inhibitory glutamate-gated chloride channels (GluCls) in the membranes of pharyngeal muscles, motor neurons, female reproductive tracts, and the excretory/secretory pores. Ivermectin increases chloride ion flow into the nerve and muscle cells, leading to paralysis and death.
Liquid chromatography-mass spectrometry (LC-MS): an analytical technique that combines liquid chromatography with mass spectrometry to separate, identify, and quantify compounds based on their mass-to-charge ratio and chromatographic behavior.
Macrocyclic lactones: a class of anthelmintic drugs characterized by large, ring-shaped molecular structures, typically containing 12 or more members. They are widely used as antiparasitic agents, particularly in veterinary and human medicine, due to their effectiveness against nematodes and arthropods.
Non-target site resistance (NTSR): a form of AR that involves changes in the organism's metabolism or drug-handling processes, such as altered drug absorption, distribution, or elimination rather than changes in the drug's target site.
Nuclear magnetic resonance (NMR) spectroscopy: an analytical technique

can reveal vulnerabilities for novel treatment approaches, such as synergistic drug combinations. By addressing both TSR and NTSR, we can better manage the growing challenge of AR.

The free-living nematode *C. elegans* is a powerful model to identify the role metabolites play in AR

Metabolomics approaches hold great potential to deepen our understanding of anthelmintic MoR, provide strategies to decrease drug inhibition to slow resistance, and improve diagnostic tools. However, the application of metabolomics methods to parasitic nematode populations faces several challenges, including limited access to relevant life cycle stages because of host dependency, the complexity of *in vitro* culture systems (e.g., adult parasitic nematodes have limited viability in culture and sufficient parasite biomass is difficult to acquire), and a lack of molecular toolkits.

The free-living nematode *C. elegans* offers solutions to these obstacles and can advance the use of metabolomics in AR research. To date, *C. elegans* has been extensively studied at cellular and genetic levels and used to study various biological processes [28–35]. *C. elegans* has been instrumental in identifying TSR mechanisms across every major anthelmintic drug class [36–43]. However, we have limited knowledge of how metabolic pathways operate in *C. elegans* and how metabolites influence the physiological responses to anthelmintic drugs.

C. elegans is an ideal model organism to examine the role of small molecules in anthelmintic drug metabolism, offering several critical advantages for metabolomics studies. First, *C. elegans* is a self-fertilizing hermaphroditic species that produces genetically identical (isogenic) offspring [28]. The genetic uniformity of *C. elegans* offspring ensures that any observed variation in metabolite profiles is caused by experimental conditions, such as drug exposure, rather than genetic

used to determine the structure and concentration of molecules in a sample by measuring the interaction of nuclear spins with an applied magnetic field. NMR provides detailed information about the molecular structure, dynamics, and chemical environment.

Target site resistance (TSR): a form of AR caused by genetic mutations that alter the drug's target site, reducing the drug's therapeutic effect.

Xenobiotic-metabolizing enzymes (XMEs): enzymes involved in the metabolism of a foreign substance (xenobiotic) such as anthelmintic drugs or toxins. XMEs facilitate the biotransformation of anthelmintic drugs, often to make them more water-soluble and easier to excrete.

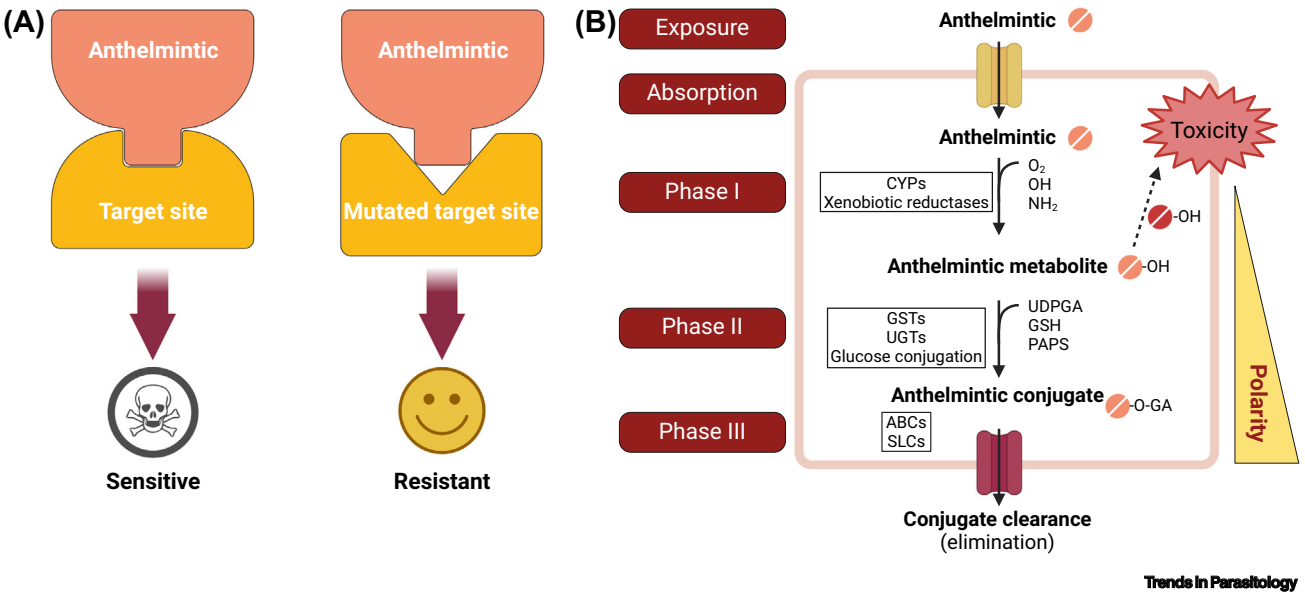

Figure 2. Target-site resistance (TSR) versus non-target-site resistance (NTSR). (A) TSR. (B) NTSR. As a change in target structure does not fully explain the whole range of resistance, other non-target site mechanisms must be involved, with NTSR based mainly on increased drug absorption, biotransformation, and elimination. For this reason, the effect of pharmacotherapy can be decreased by the enhanced inactivation and/or elimination of active drug caused by the increased expression and activity of xenobiotic-metabolizing enzymes (XMEs). The role of the XME is to protect the organism against the toxic action of drugs or other xenobiotics in three phases. Metabolic inhibitors might reduce anthelmintic biotransformation and improve drug activity. Abbreviations: ABCs, ATP-binding cassette transporters; CYPs, cytochrome P450s; GSTs, glutathione S transferases; SDRs, short-chain dehydrogenases; UGTs, UDP-glucosyltransferases. Figure created with BioRender.com.

differences among individuals [44,45]. Second, isogenic *C. elegans* strains can be grown in large numbers under standardized conditions [46–48], a necessity when performing highly variable metabolomics assays [49,50]. These growth conditions enable the use of the same sample across multiple platforms, creating the opportunity to perform multi-omics analyses [e.g., RNA-sequencing, **liquid chromatography-mass spectrometry** (**LC-MS**), and **nuclear magnetic resonance** (**NMR**) **spectroscopy**)] [46,49,50]. Third, the integration of data collected from single samples across platforms allows measurements to be incorporated with genome-scale metabolic network models that cover annotated chemical reactions in *C. elegans*, such as iCEL1314 [51], to obtain functional predictions about the role of metabolites in anthelmintic response. Fourth, the use of clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) (CRISPR-Cas9) genome editing can be employed to experimentally validate the roles of candidate metabolites in anthelmintic responses in intact animals [20,52–54]. Finally, the natural genetic variation across the *C. elegans* species is accessible and continuously archived in the *Caenorhabditis* Natural Diversity Resource (CaeNDR) [55], which has facilitated the characterization of natural responses to anthelmintic drugs and can be used to understand the natural metabolic variation in anthelmintic responses [19,56,57]. Here, we focus on how *C. elegans* can be employed to deepen our understanding of anthelmintic MoR, provide strategies to increase drug efficacy to slow resistance, and improve diagnostic tools.

Metabolomic approaches to uncover mechanisms of AR

Understanding how anthelmintic drugs are metabolized and excreted in nematodes is essential to identify the factors that influence drug efficacy, effectiveness, and MoR. However, our knowledge of xenobiotic responses and the degree to which anthelmintics are metabolized in nematodes is limited and often inconsistent. Therefore, elucidating the role of metabolism in AR is crucial to identify the limitations that affect the efficacy and spectrum of both existing drugs and novel compounds.

Among the anthelmintic drug classes, **benzimidazoles** (**BZs**) provide a particularly instructive case for exploring the complexities of drug metabolism and resistance mechanisms. The MoR to BZs are arguably the most studied and understood, which provides a valuable framework to investigate how metabolism contributes to known BZ resistance. It is well established that the main target sites for BZs are genes encoding β-tubulins, and that mutations in β-tubulin genes allow free-living and parasitic nematodes to limit the effectiveness of BZs [19,20,36,58,59]. However, alterations in the target protein alone do not account for the full range of nematode responses to BZs [19,60]. Therefore, NTSR mechanisms, including differences in drug metabolism, likely play a significant role in the observed variation in BZ drug responses.

Studies using *C. elegans* to investigate the role of NTSR mechanisms in **albendazole** (**ABZ**) response provided key insights into how these mechanisms could operate across different anthelmintic classes. Using a *C. elegans* strain with a deletion in the β-tubulin gene, *ben-1*, researchers found that when animals were exposed to ABZ they metabolized the majority of ABZ into its active metabolite ABZ sulfoxide (ABZ-SO) and two ABZ-glucoside metabolites [25]. This metabolic conversion is thought to be primarily catalyzed by flavin mono-oxygenase and the CYP3A family, with further sulfoxidation to the inactive ABZ sulfone (ABZ-SO_2), which likely occurs using the CYP1A family [25]. Like many other xenobiotics, the presence of ABZ and its metabolites has been shown to induce cytochrome P450 enzymes and other XMEs in various species [25,61–63]. Additionally, when the target site of BZs is removed, transcriptional responses in *C. elegans* are dominated by genes that encode XMEs, particularly cytochrome P450s and UDP-glucuronosyltransferases (UGTs) [25,62].

These findings are significant for two reasons. First, the involvement of XMEs in ABZ detoxification across nematode species points to additional genes and pathways that are crucial in understanding the MoR to BZs. For example, it is possible that XMEs enable the accumulation of BZs, giving nematode parasites enough time to develop resistance to BZs through β-tubulin mutations. Second, glucosylation, an uncommon metabolism pathway in mammals, has not been reported in mammalian studies of ABZ metabolism [64–67]. Hence, the metabolism of ABZ by nematodes involves the production of what could be nematode-specific metabolic processes. The differences in xenobiotic metabolism between nematodes and mammals highlight an important yet underexplored opportunity to potentiate ABZ effects by targeting these specific metabolic pathways in nematodes, thereby increasing the effective ABZ concentration.

Further research is needed to understand the role of metabolism in anthelmintic responses across nematode species and drug classes. A critical first step is to dissect the drug metabolism pathway of each anthelmintic drug, using *C. elegans*, and metabolomic profiling to map key metabolic enzymes and pathways involved in drug detoxification. Overall, identifying NTSR mechanisms is crucial to achieve a comprehensive understanding of MoR. Additionally, studying the biotransformation of drugs can reveal novel targets for the enhancement of anthelmintic efficacy, though the identification of these targets remains particularly challenging for most anthelmintic drug classes because their targets, or the full spectrum of their targets, are not yet fully identified.

Enhancing anthelmintic effectiveness: a metabolomics-based strategy to increase drug efficacy

Understanding the metabolism of anthelmintic drugs and the enzymatic pathways involved in their detoxification in nematodes is critical to identify factors that influence drug efficacy. Insights into these metabolic processes could facilitate the development of synergistic compounds to enhance the efficacy of existing anthelmintics. For instance, human cytochrome P450s and UGTs metabolize and clear more than 90% of all commercial drugs [68–70], which suggests key enzymatic targets to reduce drug inhibition and improve therapeutic outcomes. Metabolomics offers powerful approaches to identify XMEs and potential inhibitors, presenting opportunities to increase anthelmintic efficacy across populations of drug-resistant nematodes.

A growing body of evidence shows that *C. elegans* primarily metabolizes ABZ through glucose conjugation, which is also a major biotransformation pathway for BZs in the sheep parasite *Haemonchus contortus,* highlighting the translatability of *C. elegans* research to parasitic nematodes [8,25,71]. Further work showed that glucose metabolite production by *C. elegans* was reduced in the presence of the pharmacological inhibitor chrysin, which suggests that UGT enzymes might catalyze BZ glucosidation [71] (Figure 3). Beyond BZs, metabolism continues to play a critical role in our understanding of AR. When exposed to the **macrocyclic lactone ivermectin**, *C. elegans* differentially expresses genes involved in metabolism, including the cytochrome P450, *cyp-37B1* [72,73]. The nicotinic acetylcholine receptor agonist levamisole is hypothesized to lead to metabolic exhaustion in *C. elegans* [74]. Although *C. elegans* is an essential model and resource in the pipeline to identify the effects XME inhibitors have on hindering drug metabolism in nematodes and enhancing anthelmintic efficacy, it is a free-living nematode. Therefore, once we have identified and understood the general roles of nematode XMEs and XME inhibitors in anthelmintic metabolism in *C. elegans*, we must validate these effects in parasitic nematodes to confirm their relevance in these species.

The use of **enzyme inhibitors** in gastrointestinal parasitic nematodes has demonstrated increased anthelmintic drug efficacy, indicating that xenobiotic metabolism plays a significant role in resistance. In the cattle parasitic nematodes *Cooperia oncophora* and *Ostertagia ostertagi*,

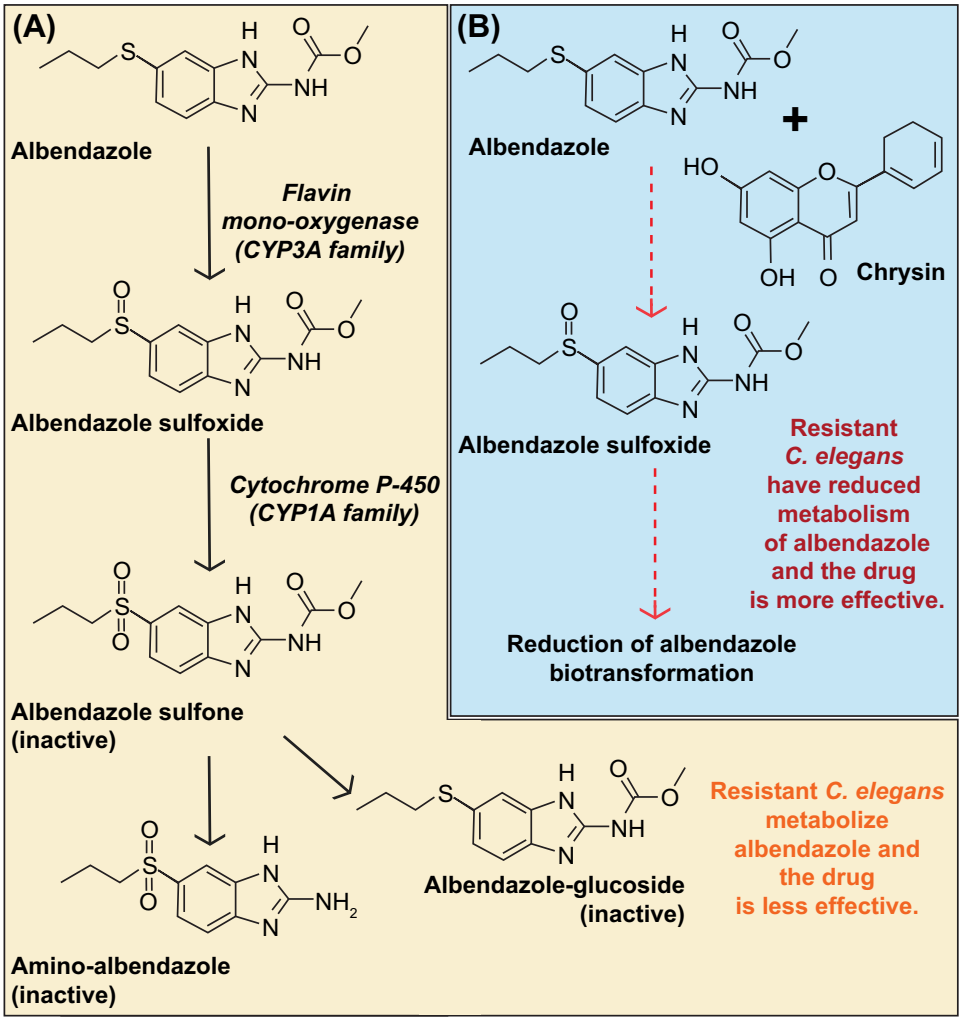

Figure 3. Chemical structure and main metabolic pathways of albendazole (ABZ). (A) Metabolic pathway of ABZ in *Caenorhabditis elegans* [25]. (B) The addition of the UDP-glucosyl transferase (UGT) inhibitor chrysin with ABZ reduced biotransformation in *C. elegans* [71].

the cytochrome P450 inhibitor piperonyl butoxide and the P-glycoprotein inhibitor verapamil completely eliminated the effects of resistance to thiabendazole and ivermectin, respectively [75,76]. These results suggest that cytochrome P450s and P-glycoproteins contribute to the metabolism of these anthelmintics [75,76]. Similarly, in *H. contortus*, the UGT inhibitor 5-nitrouracil increased the efficacy of naphthalophos (an organophosphate compound used as an anthelmintic drug in veterinary medicine) resistant nematodes in larval development assays (LDAs) [77]. Growing evidence demonstrates that XMEs significantly modulate drug efficacy, and targeting these enzymes with inhibitors could restore anthelmintic efficacy in resistant nematode populations [6,78,79].

Targeting specific metabolic pathways in nematodes is a promising strategy to enhance anthelmintic efficacy and overcome resistance. Future research should first employ metabolomics to identify and characterize XMEs in *C. elegans.* By editing genes involved in TSR, we can detect

relevant metabolic modifications, assess their impact, and then determine if these modified XMEs are conserved in parasitic nematodes. After validation in *C. elegans*, studies on parasitic nematodes could lead to the development of comprehensive strategies to manage AR, including XMEs that enable the discovery of synergistic drug combinations involving enzyme inhibitors, which could lead to more effective anthelmintic treatments. After validating XMEs and enzyme inhibitors in *C. elegans*, subsequent studies on parasitic nematodes could facilitate the development of comprehensive approaches to manage AR.

Challenges in detecting AR in helminths: can metabolomics offer a solution?

Effective management of AR requires reliable diagnostic tools to identify parasite infections and detect early signs of resistance. In cases where resistance is already widespread, diagnostic tests are critical to identify which drugs remain effective. Traditional diagnostic approaches, such as the fecal egg count reduction test (FECRT) and coproscopic techniques, have limitations. FECRT, although a routinely used method, often fails to detect resistance accurately because of a variety of factors such as the parasite species, egg-shedding rates, and host age [80–82]. Coprological methods, dependent on parasite reproductive cycles and often labor-intensive, also face challenges in diagnostic performance and sample collection [82]. Other tests, such as carbohydrate specific binding of lectins and parasite-specific antibody detection, have failed to characterize parasite species beyond *H. contortus* and unreliably identify current parasite infections, respectively [2]. Molecular diagnostics, such as PCR and metabarcoding, have improved detection capabilities but are still hindered by prior knowledge of the genetic variants, the need for specialized equipment, and processing time [83–85]. Recent advances, such as automated multiplexed-PCR platforms have been used to track parasite transmission [86] and demonstrate potential as a method to detect AR. However, significant investments are required to make automated multiplexed-PCR platforms practical for detection of AR in field populations. Finally, approaches such as COMBatting Anthelmintic Resistance in Ruminants (COMBAR) propose the use of innovative diagnostic tests [e.g., targeted (selective) treatment approaches, vaccines, antiparasitic forages] to detect helminth infections and AR [2]; however, COMBAR must implement an initiative that sets sustainable approaches and practices to reliably detect AR.

In contrast to these methods, metabolomics offers the most sensitive non-invasive approach (e.g., use of milk and urine samples) to identify biomarkers and metabolic discrepancies related to AR. Metabolomics has not been extensively used to identify biomarkers associated with AR, but it offers potential to advance our knowledge of NTSR mechanisms, essential pathways that may constitute druggable targets, and **excretory/secretory products** (**ESPs**). For example, metabolomics has revolutionized cancer biology and enabled the identification of metabolites that directly represent the molecular phenotype and profile of cancer cells [87–89], where metabolomics has led to significant advancements in clinical applications in oncology [90,91]. Additionally, metabolomics approaches have been used to characterize the *in vitro* metabolic 'footprint' of *Echinococcus multilocularis*, a fox tapeworm, where excreted or secreted metabolites (e.g., acetate, alanine, lactate, and succinate) by the parasite provide small molecules that can be explored as diagnostic target candidates [92,93]. The emerging use of metabolomics in nematode parasitology has already aided in the identification of host infection status, and similar approaches can elucidate the MoR to anthelmintics. The types of samples that can be analyzed using metabolomics are diverse and include tissues, cells, and biofluids [88,94]. By analyzing metabolites, which reflect the dynamic interplay of genetic, proteomic, and environmental factors, metabolomics can provide insights into parasite infections and the host's health status. Techniques such as LC-MS and NMR are used to profile small molecules in biological samples, offering the potential to detect infection status and AR [95,96]. Without a priori knowledge, LC-MS and NMR allow investigators to quantify known and unexpected metabolite changes in disrupted

pathways and in pathways that were not previously thought to be connected to AR [97]. Furthermore, these metabolites can be known or novel, and both can be quantified with or without the need for chemical standards [98,99]. Although NMR provides structural information and quantification of metabolites, LC-MS provides more detailed identification, quantification, and profiling with higher sensitivity, enabling the detection of a broader range of metabolites at lower concentrations. The integration of both methods can enhance the depth of metabolic analysis and improve the detection of AR.

Concluding remarks

In conclusion, the growing threat of AR requires a comprehensive, multifaceted approach that integrates insights from TSR and NTSR mechanisms. Using *C. elegans* as a model organism, we can explore both mutations that alter drug targets and metabolic pathways that neutralize drug efficacy, providing a clearer understanding of resistance mechanisms across anthelmintic classes. The potential of metabolomics to reveal key metabolic alterations and identify novel biomarkers opens new avenues to enhance the efficacy of existing treatments and develop innovative diagnostic tools (see Outstanding questions). However, these findings must be validated in parasitic nematodes to ensure translatability to field settings. For example, metabolic alterations and biomarkers of AR identified in *C. elegans* can provide lists of small molecules to identify in populations of drug-resistant and drug-sensitive parasites, such as *H. contortus* [100]. By comparing metabolite profiles between *H. contortus* populations with differing drug susceptibilities, we can associate specific metabolic changes linked to resistance and susceptibility to anthelmintic compounds.

By employing metabolomics to identify resistance mechanisms in combination with knowledge about drug metabolism, we can create new strategies, such as synergistic drug combinations and enzyme inhibitors, to restore anthelmintic efficacy and slow the progression of resistance in parasitic nematodes. This research will not only improve treatment outcomes but also provide practical solutions for managing AR in veterinary and human medicine.

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Declaration of interests

The authors declare no competing interests.

Outstanding questions

What are the specific NTSR mechanisms that contribute to AR, particularly in terms of drug metabolism and excretion across different nematodes?

How do different nematode species metabolize anthelmintic drugs, and what are the precise roles of various XMEs in these processes?

How can we overcome the limitations of current diagnostic methods, such as FECRT and molecular diagnostics, to develop more accurate, non-invasive tools for detecting AR?

Are there novel metabolic pathways and potential targets for enhancing anthelmintic efficacy?

How does variation in environmental conditions (e.g., temperature, pH, and humidity) affect AR in *C. elegans* and parasitic nematode species?

How can synergistic drug combinations or inhibitors of XMEs be used to increase anthelmintic efficacy in parasitic nematode populations?

Are the regulatory elements of genes encoding xenobiotic response elements less responsive to induction in parasitic stages compared to the free-living stages of parasites such as *Haemonchus contortus*?

Given that most commercial anthelmintics are administered in a single dose, and are fast acting, how does the time to XME induction compare with the time of parasite death? Subsequently, are XME levels high enough to contribute to AR?

References

1. Hodgkinson, J.E. *et al.* (2019) Refugia and anthelmintic resistance: concepts and challenges. *Int. J. Parasitol. Drugs Drug Resist.* 10, 1051–1057
2. Charlier, J. *et al.* (2022) Anthelmintic resistance in ruminants: challenges and solutions. *Adv. Parasitol.* 115, 171–227
3. Beech, R.N. *et al.* (2011) Anthelmintic resistance: markers for resistance, or susceptibility? *Parasitology* 138, 160–174
4. Roos, M.H. *et al.* (1990) Molecular analysis of selection for benzimidazole resistance in the sheep parasite *Haemonchus contortus*. *Mol. Biochem. Parasitol.* 43, 77–88
5. Redman, E. *et al.* (2015) The emergence of resistance to the benzimidazole anthelmintics in parasitic nematodes of livestock is characterised by multiple independent hard and soft selective sweeps. *PLoS Negl. Trop. Dis.* 9, e0003494
6. Janssen, I.J.I. *et al.* (2013) *Caenorhabditis elegans*: modest increase of susceptibility to ivermectin in individual P-glycoprotein loss-of-function strains. *Exp. Parasitol.* 134, 171–177
7. David, M. *et al.* (2018) Structural model, functional modulation by ivermectin and tissue localization of *Haemonchus contortus* P-glycoprotein-13. *Int. J. Parasitol. Drugs Drug Resist.* 8, 145–157
8. Sharma, N. *et al.* (2024) Multiple UDP glycosyltransferases modulate benzimidazole drug sensitivity in the nematode *Caenorhabditis elegans* in an additive manner. *Int. J. Parasitol.*, https://doi.org/10.1016/j.ijpara.2024.05.003
9. Burns, A.R. *et al.* (2023) Selective control of parasitic nematodes using bioactivated nematicides. *Nature* 618, 102–109
10. Page, A.P. (2018) The sensory amphidial structures of *Caenorhabditis elegans* are involved in macrocyclic lactone uptake and anthelmintic resistance. *Int. J. Parasitol.* 48, 1035–1042
11. Urdaneta-Marquez, L. *et al.* (2014) A dyf-7 haplotype causes sensory neuron defects and is associated with macrocyclic lactone resistance worldwide in the nematode parasite *Haemonchus contortus*. *Int. J. Parasitol.* 44, 1063–1071
12. Wishart, D.S. (2016) Emerging applications of metabolomics in drug discovery and precision medicine. *Nat. Rev. Drug Discov.* 15, 473–484
13. Palmer, N.D. *et al.* (2015) Metabolomic profile associated with insulin resistance and conversion to diabetes in the Insulin Resistance Atherosclerosis Study. *J. Clin. Endocrinol. Metab.* 100, E463–E468

14. Bujak, R. *et al.* (2015) Metabolomics for laboratory diagnostics. *J. Pharm. Biomed. Anal.* 113, 108–120
15. Bai, Y. *et al.* (2014) Biomarker identification and pathway analysis by serum metabolomics of childhood acute lymphoblastic leukemia. *Clin. Chim. Acta* 436, 207–216
16. Wang, J. *et al.* (2013) Metabolomic identification of diagnostic plasma biomarkers in humans with chronic heart failure. *Mol. BioSyst.* 9, 2618–2626
17. Wishart, D.S. (2008) Applications of metabolomics in drug discovery and development. *Drugs R. D.* 9, 307–322
18. Dilks, C.M. *et al.* (2021) Newly identified parasitic nematode beta-tubulin alleles confer resistance to benzimidazoles. *Int. J. Parasitol. Drugs Drug Resist.* 17, 168–175
19. Hahnel, S.R. *et al.* (2018) Extreme allelic heterogeneity at a *Caenorhabditis elegans* beta-tubulin locus explains natural resistance to benzimidazoles. *PLoS Pathog.* 14, e1007226
20. Dilks, C.M. *et al.* (2020) Quantitative benzimidazole resistance and fitness effects of parasitic nematode beta-tubulin alleles. *Int. J. Parasitol. Drugs Drug Resist.* 14, 28–36
21. Jugulam, M. and Shyam, C. (2019) Non-target-site resistance to herbicides: recent developments. *Plants* 8, 417
22. Yang, Q. *et al.* (2016) Target-site and non-target-site based resistance to the herbicide tribenuron-methyl in flixweed (*Descurainia sophia* L.). *BMC Genomics* 17, 551
23. Zgurskaya, H.I. and Nikaido, H. (2000) Multidrug resistance mechanisms: drug efflux across two membranes. *Mol. Microbiol.* 37, 219–225
24. Raza, A. *et al.* (2015) Effects of third generation P-glycoprotein inhibitors on the sensitivity of drug-resistant and -susceptible isolates of *Haemonchus contortus* to anthelmintics *in vitro*. *Vet. Parasitol.* 211, 80–88
25. Laing, S.T. *et al.* (2010) Characterization of the xenobiotic response of Caenorhabditis elegans to the anthelmintic drug albendazole and the identification of novel drug glucoside metabolites. *Biochem. J.* 432, 505–514
26. Tedla, B.A. *et al.* (2019) Novel cholinesterase paralogs of *Schistosoma mansoni* have perceived roles in cholinergic signalling and drug detoxification and are essential for parasite survival. *PLoS Pathog.* 15, e1008213
27. Ménez, C. *et al.* (2019) The transcription factor NHR-8: a new target to increase ivermectin efficacy in nematodes. *PLoS Pathog.* 15, e1007598
28. Brenner, S. (1974) The genetics of *Caenorhabditis elegans*. *Genetics* 77, 71–94
29. Bargmann, C.I. (1998) Neurobiology of the *Caenorhabditis elegans* genome. *Science* 282, 2028–2033
30. Fire, A. *et al.* (1998) Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* 391, 806–811
31. Link, C.D. *et al.* (2003) Gene expression analysis in a transgenic *Caenorhabditis elegans* Alzheimer's disease model. *Neurobiol. Aging* 24, 397–413
32. Lui, D.Y. and Colaiácovo, M.P. (2013) Meiotic development in *Caenorhabditis elegans*. *Adv. Exp. Med. Biol.* 757, 133–170
33. Yuan, J. *et al.* (1993) The *C. elegans* cell death gene ced-3 encodes a protein similar to mammalian interleukin-1 beta-converting enzyme. *Cell* 75, 641–652
34. Vella, M.C. and Slack, F.J. (2005) C. elegans microRNAs. In *WormBook: The Online Review of C. elegans Biology [Internet]*, pp. 2005–2018, WormBook, Pasadena (CA)
35. C. elegans Sequencing Consortium (1998) Genome sequence of the nematode *C. elegans:* a platform for investigating biology. *Science* 282, 2012–2018
36. Driscoll, M. *et al.* (1989) Genetic and molecular analysis of a *Caenorhabditis elegans* beta-tubulin that conveys benzimidazole sensitivity. *J. Cell Biol.* 109, 2993–3003
37. Dent, J.A. *et al.* (2000) The genetics of ivermectin resistance in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. U. S. A.* 97, 2674–2679
38. Lewis, J.A. *et al.* (1980) The genetics of levamisole resistance in the nematode *Caenorhabditis elegans*. *Genetics* 95, 905–928
39. Fleming, J.T. *et al.* (1997) *Caenorhabditis elegans* levamisole resistance geneslev-1, unc-29, and unc-38 encode functional nicotinic acetylcholine receptor subunits. *J. Neurosci.* 17, 5843–5857
40. Towers, P.R. *et al.* (2005) The *Caenorhabditis elegans* lev-8 gene encodes a novel type of nicotinic acetylcholine receptor alpha subunit. *J. Neurochem.* 93, 1–9
41. Culetto, E. *et al.* (2004) The *Caenorhabditis elegans* unc-63 gene encodes a levamisole-sensitive nicotinic acetylcholine receptor alpha subunit. *J. Biol. Chem.* 279, 42476–42483
42. Kaminsky, R. *et al.* (2008) A new class of anthelmintics effective against drug-resistant nematodes. *Nature* 452, 176–180
43. Guest, M. *et al.* (2007) The calcium-activated potassium channel, SLO-1, is required for the action of the novel cyclooctadepsipeptide anthelmintic, emodepside, in *Caenorhabditis elegans*. *Int. J. Parasitol.* 37, 1577–1588
44. Fox, B.W. *et al.* (2022) *C. elegans* as a model for inter-individual variation in metabolism. *Nature* 607, 571–577
45. Corsi, A.K., *et al.* A Transparent window into biology: a primer on *Caenorhabditis elegans*. In: *WormBook: The Online Review of C. elegans Biology [Internet]*. Pasadena (CA): WormBook; 2005–2018. Available from: https://www.ncbi.nlm.nih.gov/books/NBK299460/
46. Shaver, A.O. *et al.* (2021) Culture and assay of large-scale mixed-stage *Caenorhabditis elegans* populations. *J. Vis. Exp.* 171
47. Hibshman, J.D. *et al.* (2021) Liquid-culture protocols for synchronous starvation, growth, dauer formation, and dietary restriction of *Caenorhabditis elegans*. *STAR Protoc.* 2, 100276
48. Lewis, J.A. and Fleming, J.T. (1995) Chapter 1 Basic Culture Methods. In *Methods in Cell Biology* (48) (Epstein, H.F. and Shakes, D.C., eds), pp. 3–29, Academic Press
49. Shaver, A.O. *et al.* (2022) An anchored experimental design and meta-analysis approach to address batch effects in large-scale metabolomics. *Front. Mol. Biosci.* 9, 930204
50. Gouveia, G.J. *et al.* (2021) Long-term metabolomics reference material. *Anal. Chem.* 93, 9193–9199
51. Yilmaz, L.S. and Walhout, A.J.M. (2016) A *Caenorhabditis elegans* genome-scale metabolic network model. *Cell Syst.* 2, 297–311
52. Evans, K.S. *et al.* (2021) From QTL to gene: *C. elegans* facilitates discoveries of the genetic mechanisms underlying natural variation. *Trends Genet.* 37, 933–947
53. Ghosh, R. *et al.* (2012) Natural variation in a chloride channel subunit confers avermectin resistance in *C. elegans*. *Science* 335, 574–578
54. Frøkjær-Jensen, C. (2013) Exciting prospects for precise engineering of *Caenorhabditis elegans* genomes with CRISPR/Cas9. *Genetics* 195, 635–642
55. Crombie, T.A. *et al.* (2024) CaeNDR, the Caenorhabditis Natural Diversity Resource. *Nucleic Acids Res.* 52, D850–D858
56. Shaver, A.O. *et al.* (2022) Variation in anthelmintic responses are driven by genetic differences among diverse *C. elegans* strains. *PLoS Pathog.* 19, e1011285
57. Evans, K.S. *et al.* (2021) Two novel loci underlie natural differences in *Caenorhabditis elegans* abamectin responses. *PLoS Pathog.* 17, e1009297
58 Pallotto, L.M. *et al.* (2022) Interactions of *Caenorhabditis elegans* β-tubulins with the microtubule inhibitor and anthelmintic drug albendazole. *Genetics* 221, iyac093
59. Shaver, A.O. *et al.* (2024) Quantifying the fitness effects of resistance alleles with and without anthelmintic selection pressure using *Caenorhabditis elegans*. *PLoS Pathog.* 20, e1012245
60. Hahnel, S.R. *et al.* (2020) *Caenorhabditis elegans* in anthelmintic research – old model, new perspectives. *Int. J. Parasitol. Drugs Drug Resist.* 14, 237–248
61. Pech-Santiago, E.O. *et al.* (2022) *Giardia duodenalis*: flavohemoglobin is involved in drug biotransformation and resistance to albendazole. *PLoS Pathog.* 18, e1010840
62. Laing, R. *et al.* (2015) The cytochrome P450 family in the parasitic nematode *Haemonchus contortus*. *Int. J. Parasitol.* 45, 243–251
63. Cvilink, V. *et al.* (2008) LC–MS–MS identification of albendazole and flubendazole metabolites formed *ex vivo* by *Haemonchus contortus*. *Anal. Bioanal. Chem.* 391, 337–343
64. Delatour, P. *et al.* (1991) Comparative enantioselectivity in the sulphoxidation of albendazole in man, dogs and rats. *Xenobiotica* 21, 217–221

65. Delatour, P. *et al.* (1991) Chiral behaviour of the metabolite albendazole sulphoxide in sheep, goats and cattle. *Res. Vet. Sci.* 50, 134–138
66. Moroni, P. *et al.* (1995) Chiral sulfoxidation of albendazole by the flavin adenine dinucleotide-containing and cytochrome P450-dependent monooxygenases from rat liver microsomes. *Drug Metab. Dispos.* 23, 160–165
67. Theurillat, R. *et al.* (2021) Aspects of albendazole metabolism in western lowland gorillas (*Gorilla gorilla gorilla*) compared to humans and other species assessed by HPLC, LC-MS, and chiral electrokinetic chromatography. *Sep. Sci. Plus* 4, 347–356
68. Rowland, A. *et al.* (2013) The UDP-glucuronosyltransferases: their role in drug metabolism and detoxification. *Int. J. Biochem. Cell Biol.* 45, 1121–1132
69. Zanger, U.M. and Schwab, M. (2013) Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. *Pharmacol. Ther.* 138, 103–141
70. Mazerska, Z. *et al.* (2016) The role of glucuronidation in drug resistance. *Pharmacol. Ther.* 159, 35–55
71. Stasiuk, S.J. *et al.* (2019) Similarities and differences in the biotransformation and transcriptomic responses of *Caenorhabditis elegans* and *Haemonchus contortus* to five different benzimidazole drugs. *Int. J. Parasitol. Drugs Drug Resist.* 11, 13–29
72. Dube, F. *et al.* (2022) Ivermectin-induced gene expression changes in adult *Parascaris univalens* and *Caenorhabditis elegans*: a comparative approach to study anthelminthic metabolism and resistance *in vitro*. *Parasit. Vectors* 15, 158
73. Laing, S.T. *et al.* (2012) The transcriptional response of *Caenorhabditis elegans* to Ivermectin exposure identifies novel genes involved in the response to reduced food intake. *PLoS One* 7, e31367
74. Chaya, T. *et al.* (2021) A *C. elegans* genome-wide RNAi screen for altered levamisole sensitivity identifies genes required for muscle function. *G3* 11, jkab047
75. AlGusbi, S. *et al.* (2014) Analysis of putative inhibitors of anthelmintic resistance mechanisms in cattle gastrointestinal nematodes. *Int. J. Parasitol.* 44, 647–658
76. Demeler, J. *et al.* (2013) Potential contribution of P-glycoproteins to macrocyclic lactone resistance in the cattle parasitic nematode *Cooperia oncophora*. *Mol. Biochem. Parasitol.* 188, 10–19
77. Kotze, A.C. *et al.* (2014) Phenobarbital induction and chemical synergism demonstrate the role of UDP-glucuronosyltransferases in detoxification of naphthalophos by *Haemonchus contortus* larvae. *Antimicrob. Agents Chemother.* 58, 7475–7483
78. Gerhard, A.P. *et al.* (2020) The P-glycoprotein repertoire of the equine parasitic nematode *Parascaris univalens*. *Sci. Rep.* 10, 13586
79. Kerboeuf, D. *et al.* (2003) P-glycoprotein in helminths: function and perspectives for anthelmintic treatment and reversal of resistance. *Int. J. Antimicrob. Agents* 22, 332–346
80. Morgan, E.R. *et al.* (2022) Confounding factors affecting faecal egg count reduction as a measure of anthelmintic efficacy. *Parasite* 29, 20
81. Kaplan, R.M. and Vidyashankar, A.N. (2012) An inconvenient truth: global worming and anthelmintic resistance. *Vet. Parasitol.* 186, 70–78
82. Kaplan, R.M. *et al.* (2023) World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) guideline for diagnosing anthelmintic resistance using the faecal egg count reduction test in ruminants, horses and swine. *Vet. Parasitol.* 318, 109936
83. Kotze, A.C. *et al.* (2014) Recent advances in candidate-gene and whole-genome approaches to the discovery of anthelmintic resistance markers and the description of drug/receptor interactions. *Int. J. Parasitol. Drugs Drug Resist.* 4, 164–184
84. Doyle, S.R. and Cotton, J.A. (2019) Genome-wide approaches to investigate anthelmintic resistance. *Trends Parasitol.* 35, 289–301
85. Kotze, A.C. *et al.* (2020) Challenges and opportunities for the adoption of molecular diagnostics for anthelmintic resistance. *Int. J. Parasitol. Drugs Drug Resist.* 14, 264–273
86. Alba, A. *et al.* (2015) A multiplex PCR for the detection of *Fasciola hepatica* in the intermediate snail host *Galba cubensis*. *Vet. Parasitol.* 211, 195–200
87. Danzi, F. *et al.* (2023) To metabolomics and beyond: a technological portfolio to investigate cancer metabolism. *Signal Transduct. Target. Ther.* 8, 137
88. Chetwynd, A.J. *et al.* (2017) Collection and preparation of clinical samples for metabolomics. *Adv. Exp. Med. Biol.* 965, 19–44
89. Schmidt, D.R. *et al.* (2021) Metabolomics in cancer research and emerging applications in clinical oncology. *CA Cancer J. Clin.* 71, 333–358
90. Beger, R.D. (2013) A review of applications of metabolomics in cancer. *Metabolites* 3, 552–574
91. Puchades-Carrasco, L. and Pineda-Lucena, A. (2017) Metabolomics applications in precision medicine: an oncological perspective. *Curr. Top. Med. Chem.* 17, 2740–2751
92. Ritler, D. *et al.* (2019) In vitro metabolomic footprint of the *Echinococcus multilocularis* metacestode. *Sci. Rep.* 9, 19438
93. Papaiakovou, M. *et al.* (2022) Worms and bugs of the gut: the search for diagnostic signatures using barcoding, and metagenomics–metabolomics. *Parasit. Vectors* 15, 118
94. Johnson, C.H. *et al.* (2016) Metabolomics: beyond biomarkers and towards mechanisms. *Nat. Rev. Mol. Cell Biol.* 17, 451–459
95. Guidi, A. *et al.* (2020) Drug effects on metabolic profiles of *Schistosoma mansoni* adult male parasites detected by 1H-NMR spectroscopy. *PLoS Negl. Trop. Dis.* 14, e0008767
96. Sallé, G. *et al.* (2021) Integrative biology defines novel biomarkers of resistance to strongylid infection in horses. *Sci. Rep.* 11, 14278
97. Edison, A.S. *et al.* (2021) NMR: unique strengths that enhance modern metabolomics research. *Anal. Chem.* 93, 478–499
98 Mashruwala, A.A. *et al.* (2019) The ClpCP complex modulates respiratory metabolism in *Staphylococcus aureus* and is regulated in a SrrAB-dependent manner. *J. Bacteriol.* 201, e00188-19
99. Marshall, D.D. *et al.* (2016) Redox imbalance underlies the fitness defect associated with inactivation of the PTA-AckA pathway in *Staphylococcus aureus*. *J. Proteome Res.* 15, 1205–1212
100. Doyle, S.R. *et al.* (2020) Genomic and transcriptomic variation defines the chromosome-scale assembly of *Haemonchus contortus*, a model gastrointestinal worm. *Commun. Biol.* 3, 656