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Ivermectin as a novel malaria control tool: Getting ahead of the resistance curse

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ABSTRACT

Reduction in malaria clinical cases is strongly dependent on the ability to prevent *Anopheles* infectious bites. Vector control strategies using long-lasting insecticidal nets and indoor residual spraying with insecticides have contributed to significantly reduce the incidence of malaria in many endemic countries, especially in the Sub-Saharan region. However, global progress in reducing malaria cases has plateaued since 2015 mostly due to the increased insecticide resistance and behavioral changes in *Anopheles* vectors. Additional control strategies are thus required to further reduce the burden of malaria and contain the spread of resistant and invasive *Anopheles* vectors. The use of endectocides such as ivermectin as an additional malaria control tool is now receiving increased attention, driven by its different mode of action compared to insecticides used so far and its excellent safety record for humans. In this opinion article, we discuss the advantages and disadvantages of using ivermectin for malaria control with a focus on the risk of selecting ivermectin resistance in malaria vectors. We also highlight the importance of understanding how ivermectin resistance could develop in mosquitoes and what its underlying mechanisms and associated molecular markers are, and propose a research agenda to manage this phenomenon.

1. The need for alternative vector control strategies

Preventing *Anopheles* mosquito bites is essential for controlling malaria infection. In areas where the main malaria vector species are endophagic (*i.e.* prefer to bite indoors), endophilic (*i.e.* prefer to rest indoors) and anthropophilic (*i.e.* prefer to feed on humans), the use of long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) with insecticides have been shown to be particularly effective. These two interventions take advantage of the behavior of major malaria vectors, and have become the pillars of malaria control (Killeen, 2014; Russell et al., 2013). In the context of renewed efforts against malaria, an estimated 663 million cases were averted between 2000 and 2015, of which 68% were attributed to the use of LLINs and 10% to IRS (Bhatt et al., 2015). This remarkable reduction in the burden of malaria was achieved thanks to a strong multilateral commitment and an increased international financing effort for malaria control (Pigott et al., 2012).

List of abbreviations: ABC-transporter, Adenosine Triphosphate-Binding Cassette transporter; Ace1, Acetylcholinesterase 1; CYP, cytochrome P450; DDT, Dichlorodiphenyltrichloroethane; GABA, Gamma-aminobutyric acid; GAL4/UAS, Gal4/upstream activating sequence; GluCl, Glutamate-gated chloride channel; GST, Glutathione S-Transferases; IRS, Insecticide Residual Spraying; IVM, Ivermectin; LC, Lethal Concentration; LLIN, Long-Lasting Insecticidal Nets; MDA, Mass Drug Administration; NTD, Neglected Tropical Diseases; PBO, Piperonyl butoxide; P-gp, P-glycoprotein; VGSC, Voltage-Gated Sodium Channel; WHO, World Health Organization.

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This significant public health achievement sparked enthusiasm to continue the control efforts with the goal of elimination in areas where LLINs and IRS were highly successful.

However, the 2021 world malaria report (World Health Organization, 2021) indicates that global progress in reducing malaria cases has stalled since 2015, with less than 2% decline in malaria incidence between 2015 and 2020, and even an increase in several countries including Eritrea, Namibia, Angola, Botswana, Burundi, the Comoros and Madagascar. The development of effective insecticide-based vector control strategies is increasingly challenging because of the widespread emergence of insecticide resistance in malaria vectors. Until 2017, pyrethroids were the only class of insecticides recommended by the World Health Organization (WHO) for impregnating mosquito nets (Gavi, 2022). This large-scale use of pyrethroids in public health in addition to their use in agriculture has selected among mosquito populations, individuals bearing resistance mechanisms such as target site mutations, cuticular resistance, and metabolic resistance (Balabanidou et al., 2016; Edi et al., 2014; Martinez-Torres et al., 1998; Wood et al., 2010; Yahouédo et al., 2017). Insecticide resistance has become so widespread across malaria endemic countries that it became obvious to the scientific community that LLINs and IRS alone would not be sufficient to reach elimination even if they were efficiently implemented by malaria control programs (Loha et al., 2019).

Moreover, while insecticide resistance in mosquitoes is primarily seen as the capacity to survive the exposure to established lethal concentrations, behavioral changes leading to reduced exposure or full avoidance can also significantly hamper insecticide efficacy. These behavioral changes include a shift in resting (*i.e.* exophily) and feeding preferences (*i.e.* exophagy and zoophagy) (Avila et al., 2021; Fornadel et al., 2010; Moiroux et al., 2012; Moreno et al., 2015; Reddy et al., 2011; Russell et al., 2016; Sougoufara et al., 2014), early (before dusk) or late (after dawn) biting activities (Avila et al., 2021; Cooke et al., 2015; Moiroux et al., 2012; Yohannes and Boelee, 2012) and daytime biting activities (Sangbakembi-Ngounou et al., 2022) as well as early exit from households after feeding (Killeen et al., 2017). These changes occurred in presence of strong selective pressure with over two billion nets delivered in 2020 (USAID, 2020) and involved either ecological shifts in vector species composition (Bugoro et al., 2011; Gillies and Smith, 1960; Mwangangi et al., 2013; Sougoufara et al., 2016) or evolutionary adaptations of vector species aggressive behaviors (Carrasco et al., 2019) that are increasingly documented in malaria endemic countries. These departures from canonical behaviors together with physiological resistance represent loopholes in the effectiveness of the current integrated vector control strategy that exclusively relies on LLINs and IRS. The direct consequence of these changes in the persistence of malaria transmission (Carnevale and Manguin, 2021; Sherrard-Smith et al., 2019).

This persistence of malaria transmission despite efficient use of current vector control strategies is termed residual transmission. This phenomenon defines the limits of what is achievable with currently available vector control tools and threatens the achievement of malaria elimination goals. Additional, complementary strategies to LLINs and/or IRS are urgently required to further reduce the burden of malaria and contain the spread of physiologically and/or behaviorally resistant as well as invasive vectors (Churcher et al., 2016; Feachem et al., 2019; Riveron et al., 2016).

2. Ivermectin as a complementary tool to control malaria

The use of endectocides to increase the mortality rate of female mosquitoes before they can transmit the malaria parasite is a promising approach to reduce malaria transmission (Macdonald, 1952). Endectocides are chemicals that were first widely used in the livestock industry to control endoparasites (*i.e.* intestinal nematodes) and ectoparasites (*i.e.* blood-feeding arthropods) (Burg et al., 1979). Several endectocide drugs are effective against a wide range of endo- and ectoparasites in animals as well as in humans, including the avermectins (abamectin, doramectin, eprinomectin, and ivermectin) and the milbemycins (milbemectin, moxidectin, nemadectin) (Floate, 2006). Ivermectin was the first endectocide used in humans, and it has been licensed since 1987 as a microfilaricide for the control of human endemic helminthiases (onchocerciasis and lymphatic filariasis) through mass drug administration (MDA) (Amazigo et al., 2002; Boatin, 2008). Interestingly, malaria vectors have been shown to be sensitive to therapeutic doses of ivermectin when feeding on treated people or animals (Derua et al., 2015; Lyimo et al., 2017; Makhanthisa et al., 2021; Pooda et al., 2015). Thus, this molecule could be considered as an “systemic insecticide” directly distributed by treated vertebrate hosts, including humans (Fig. 1).

2.1. Some advantages

Unlike pyrethroid insecticides that primarily target the voltage-gated sodium channels, ivermectin binds to the glutamate-gated chloride channels (GluCls) which regulate the passage of chloride ions into and out of the invertebrate neuronal or muscular cells. By hyper- activating the channels, ivermectin induces muscle paralysis and flaccid death (Ikeda, 2003). Glucls are not present in vertebrates, and although cross reaction with GABA-gated chloride channels is possible, the latter are present in the central nervous system and protected by the blood brain barrier that actively pumps out xenobiotics, hence ivermectińs excellent safety track record in humans and livestock (Chaccour et al., 2013). Carried by the host himself, ivermectin administered through MDAs to humans and/or animals will likely target malaria vectors regardless of their temporal or spatial feeding behavior (Fig. 1). Ivermectin has the potential to decrease malaria transmission by reducing the survival as well as the fecundity of malaria vector mosquitoes (reviewed by (Khaligh et al., 2021; Singh and Singh, 2021)) and the impact could be greater in highly seasonal (about four months) and seasonal malaria transmission settings. Indeed, a modeling study based on epidemiological data from Senegal, Liberia and Burkina Faso has predicted that ivermectin MDA in human administered in three monthly rounds, each with three consecutive daily doses of 300 μg/kg and reaching 70% coverage could reduce malaria clinical incidence by 71% and prevalence by 34% in highly seasonal moderate transmission settings (Slater et al., 2020). The authors also predict that adding ivermectin MDA to seasonal malaria chemoprevention in these settings would reduce clinical incidence by an additional 77% in children younger than 5 years compared with seasonal malaria chemoprevention alone (Slater et al., 2020). Various trials are evaluating ivermectin MDA as a complementary malaria vector control tool (Table 1). All these studies will bring an in-depth insight of the potential of ivermectin to become a valuable tool to be added in the malaria control toolbox.

Aside its impact on adult *Anopheles* survival and fecundity, ivermectin present in larval habitats can also reduce the survival of larvae of *An. gambiae s.l.* (Derua et al., 2016). Indeed, the dissipation half-life 50 (DT50, which is the time required for the concentration to decline to half of the initial value) of ivermectin is variable ranging from 16 to 458 days in soil, depending on soil type, sorption capacity, temperature, and oxygen availability (Krogh et al., 2009), and from <1 to 127 days in water-sediment systems (Löffler et al., 2005; Prasse et al., 2009). This suggests that ivermectin that is excreted in the environment after MDA to humans or animals could persist in the environment (Liebig et al., 2010) and indirectly affect malaria vector populations, and thereby malaria transmission. In addition to its toxicity for malaria vectors, ivermectin, even at sub-lethal concentrations, may inhibit the sporogony of *P. falciparum* and *P. vivax* in malaria vectors (Kobylinski et al., 2017, 2012). Additional benefits may come from the effect of ivermectin on other endo- and ectoparasites (Dourmishev et al., 2005; Nuesch et al., 2005); MDA of ivermectin for malaria could reduce the burden of intestinal and ectoparasites (Crump, 2017).

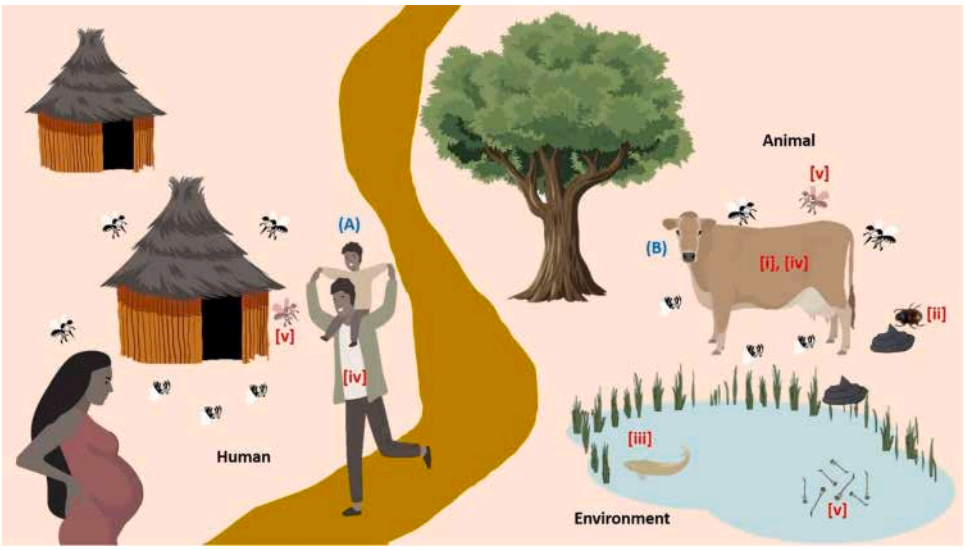

Fig. 1. Schematic representation of the use of ivermectin to combat malaria in a One Health approach and its potential ecological impacts. *Residual malaria transmission could be addressed by administering ivermectin to human* ***(A)*** *and/or to livestock* ***(B)*** *through MDAs. In addition of targeting malaria mosquitoes and animal ectoparasites, the drug will improve human and animal health by targeting intestinal parasites. However, the drug can accumulate in animal meat and milk* ***[i]*** *making them temporarily unfit for human consumption. Ivermectin and its metabolites are mainly excreted through feces. Fecal residues of ivermectin in cattle dung may affect dung-dwelling insects such as dung beetles and flies,* ***[ii]*** *leading to less amended soils. Some portions can also contaminate waterbodies and affect invertebrate and aquatic organisms* ***[iii]****. The repeated use of ivermectin has resulted in the spread of resistance mechanisms due to the strong selective pressure on herd parasites* ***[iv]****, a process that can likely occurs in human parasites and mosquito populations (larva and adults)* ***[v]****.*

Table 1
Previous and ongoing trials assessing the efficacy of ivermectin as a complementary malaria control tool.

Study name	Lead researchers	Country	Study design	Number of participants	Dose	Regimen	MDA frequency	Treated host	Study period
REACT I	Cedric Pennetier and Roch Dabiré	Burkina Faso	cRCT	2609	200 µg/kg	Single dose	Every 4 weeks for 4 consecutive months	Livestock	2016–2018
RIMDAMAL I (Foy et al., 2019)	Brian Foy and Roch Dabiré	Burkina Faso	cRCT	2712	150–200 µg/kg	Single dose	Every 3 weeks for 4 consecutive months	Human	2014–2016
RIMDAMAL II	Brian Foy and Roch Dabiré	Burkina Faso	cRCT	4088	300 µg/kg	X 3 consecutive days	X 4 consecutive months	Human	2018–2022
ANNIVERMATE (Pooda et al., 2023)	Karine Mouline	Burkina Faso	Experimental	8	1.2 mg/kg (*long-acting formulation*)	Single dose	Once	Livestock	2018–2020
MATAMAL	Anna Last	Guinea-Bissau	cRCT	24,000	300 µg/kg + DHA-P	X 3 consecutive days	X 3 consecutive months	Human	2019–2022
IVERMECTIN MDA	Jetsumon Prachumsri	Thailand	cRCT	6356	400 µg/kg	Single dose	X 3 consecutive months	Human	2017–2023
BOHEMIA	Regina Rabinovich and Carlos Chaccour	Mozambique and Kenya	cRCT	20,000–22,000	400 µg/kg	Single dose	X 3 consecutive months	Human and/or livestock	2019–2024
IMPACT	Karine Mouline	Burkina Faso	Experimental	36	0.6–1.5 mg/kg (*long-acting formulation*)	Single dose	Once	Livestock	2020–2023
MASSIV (Dabira et al., 2022)	Umberto D'alessandro	The Gambia	cRCT	4939	300–400 µg/kg + DHA-P	X 3 consecutive days	X 3 consecutive months	Human	2017–2020

cRCT= cluster Randomized Controlled Trial; DHA-*P* = Di-Hydro-Artemisinin–Piperaquine;.

2.2. Some challenges

A broader use of ivermectin to control malaria can pose several challenges including, potential environmental contamination and effects on non-target organisms (Floate, 2006), withdrawal times in livestock and implications milk or meat production (Chaccour, 2021), risk of inducing resistance in livestock or human parasites; Eng and Prichard, 2005; Schwab et al., 2007) (Fig. 1).

Ivermectin is poorly metabolized in both humans and animals. Almost 80 to 98% of the administered dose of ivermectin and/or its metabolites are excreted in the feces (Herd et al., 1996). The total amount of ivermectin present in the environment will thus depend on the number of treated human or livestock in an area, manure management and other agricultural practices. Repeated MDA of ivermectin to humans and animals can pose a risk of contamination of freshwater systems from agriculture or latrines through runoff, groundwater seepage or direct deposition (Halley et al., 1989; Nessel et al., 1989). Mosquito breeding sites could also be contaminated by ivermectin, exposing *Anopheles* larvae to the drug (Fig. 1). While some studies have shown that *Anopheles* larvae are susceptible to ivermectin, repeated exposure of aquatic life stages to ivermectin could make malaria vectors at adult stage less susceptible to ivermectin as it is the case for current

insecticides used both for agriculture and vector control (Nkya et al., 2014; Urio et al., 2022). However, selection pressure of ivermectin at larval stages may differ from the one caused by blood intake from ivermectin-treated hosts. This may select for different resistance phenotypes, albeit this has not been thoroughly investigated so far.

3. Resistance to ivermectin in mosquitoes and other arthropods

3.1. Theoretical framework of ivermectin resistance selection

Random genetic mutations that occur during reproduction cause the offspring to vary in some way from their parents (Lederberg and Lederberg, 1952). These changes that may be damaging can sometimes give an advantage to an individual. A favorable mutation may increase an individual's chances of surviving a harmful factor (*i.e.* drug, insecticide) long enough to reproduce and pass this new trait on to its offspring. If the drug or insecticide is applied intensively and repeatedly, successive populations will become less and less susceptible to the drug or insecticide. Individuals that are not removed any more by the drug or insecticide used according to the label recommendation for that individuals are referred to as "resistant or mutant".

Ivermectin is efficacious against most drug-naïve endoparasites at doses as low as 20 μg/kg body weight (Shoop, 1993), but doses of 150–200 μg/kg are needed to target the less susceptible parasites in its spectrum/label, (the **dose-defining species**). This allows for an interesting phenomenon to occur called the **window of scalation** in which the most susceptible species in the spectrum of the drug could become resistant, requiring higher and higher concentration for its management, and yet this would not be perceived until resistance has grown 10-fold (from 20 to 200 μg/kg) given that the dose used was initially defined by the less susceptible species.

Resistance in livestock parasites (*i.e.* sheep, horses and cattle roundworms) has emerged shortly after large scale, intensive use of ivermectin for veterinarian purposes (Campbell et al., 1983; Shoop, 1993). Surprisingly, reports of resistance of ivermectin in arthropods remain scarce with only two ivermectin-resistant arthropods (scabies mites and head lice) reported from human infestations in field situation (Currie et al., 2004; Diatta et al., 2016). In addition, despite over 30 years of ivermectin MDAs to control neglected tropical diseases (NTDs) (Lawrence et al., 2015), no obvious evidence of resistance to ivermectin has been seen in mosquitoes to date. Several factors could contribute to this: MDAs for NTDs are carried out only once per year, only females are exposed to the drug in adult stage and ivermectin has a well characterized impact in mosquito fertility (see **window of selection** below). This could have avoided the development and spread of ivermectin resistance in mosquito populations, although limited testing and the lack of a relevant surveillance system hinders the ability to draw conclusions from this lack of evidence.

In mosquitoes, a favorable genetic mutation could appear on either a single individual or a geographical area (*i.e.* a majority of mosquitoes in a village). The high concentration of ivermectin in the treated hosts' (humans or animals) bloodstream last for a few days after treatment killing both wild type and mutant mosquitoes. However, the effective concentration of ivermectin declines after 7 days post-treatment (Lyimo et al., 2017; Makhanthisa et al., 2021). Mutant mosquitoes exposed to lower concentrations could survive the exposure of ivermectin while the most susceptible ones are killed. The period of time after treatment within which mutant individuals can thrive while the susceptible ones are suppressed is termed the **window of selection** (Stepniewska and White, 2008). The window of selection of ivermectin opens when the concentration of the drug is low enough to allow mutant individuals to reproduce but high enough to suppress the development of susceptible ones, and closes once both mutant and susceptible individuals have equal survival probabilities (Fig. 2). Unlike insecticides used for IRS and on LLINs whose windows of selection continue to open for many months or years (South et al., 2019), that of ivermectin is probably narrower,

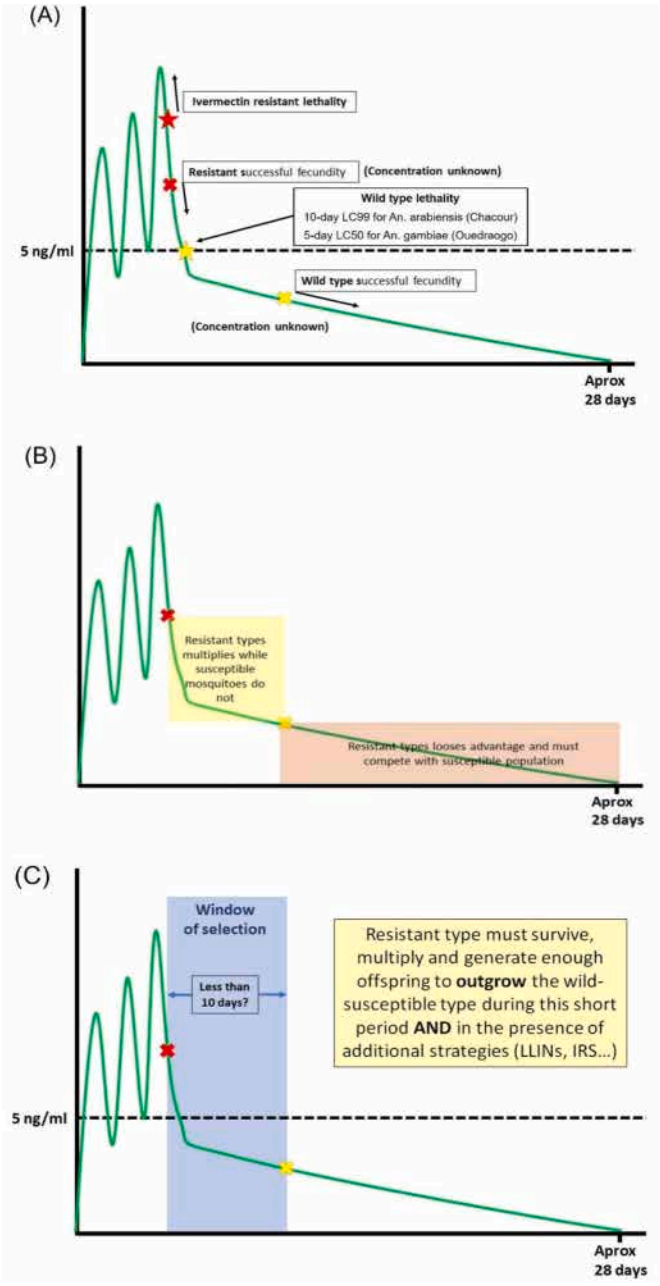

Fig. 2. Window of selection of ivermectin resistance in mosquitoes Three doses of ivermectin are given 24 h apart each. The efficacious concentration 50 (or (Lethal Concentration 50, LC50) is 5 ng/ml which kills 50% of An. gambiae in 5 days. Ivermectin also precludes mosquito reproduction at lower concentrations. If a resistant type appears, there will be a temporal gap between the concentration inhibiting the fecundity of the resistant and the wild type. Stars indicate wild type (yellow) and mutant (red) 5 day-LC50, while crosses indicate wild type (yellow) and mutant (red) concentrations allowing successful fecundity (concentration unknown), **(A)**. During the time the average ivermectin concentration is between these two concentrations in the population, the resistant type can thrive while the susceptible type is suppressed, **(B)**. The window of selection is relatively narrow for ivermectin and mosquitoes, particularly when one considers the potential concurrent presence of other vector control tools such as LLINs and IRS, **(C)**.

persisting only for a few days or weeks, depending on the dose and/or formulation. Repetitive MDAs of ivermectin to control malaria would theoretically increase the risk of selection by opening sequential windows of selection, allowing resistant phenotype to amplify and be successfully passed onward. However, ivermectin has the advantage of

reducing not only the survival and fecundity of exposed *Anopheles*, but also their locomotor activity (Sampaio et al., 2017). This may decrease the efficiency of blood-feeding, reducing the ability of mutant individuals to quickly amplify. Yet, it does not bring the risk of emergence and spread of ivermectin resistance to zero since larvae, pupae and adult emergence have been reported (at low rates) in wild and laboratory *Anopheles* mosquitoes at a low concentration of ivermectin (Eba et al., 2023).

3.2. Physiological resistance

Numerous mechanisms has been associated with ivermectin resistance in arthropods including reduced cuticular penetration in larvae (Chen et al., 2016) mutation of the targeted GluCl (Amanzougaghene et al., 2018; Diatta et al., 2016; Kwon et al., 2010; Wang et al., 2017, 2016) and metabolic through the overexpression of xenobiotic pumps (Luo et al., 2013; Mangia et al., 2016; Pohl et al., 2011; Yoon et al., 2011) and cytochrome P450 isoenzymes (Gao et al., 2016; Le Gall et al., 2018; Nicolas et al., 2021).

The GluCl channel is the primary target of ivermectin in arthropods (Cully et al., 1994). In the malaria vector *An. gambiae*, the alternative splicing process during gene expression that allows a single gene to code for multiple proteins can lead to the production of ivermectin-sensitive or -insensitive homomultimers (Meyers et al., 2015). This suggests that resistance to ivermectin could arise through altered regulation of the GluCl splicing in *An. gambiae* and probably in other *Anopheles* species as well. Furthermore, metabolic resistance mechanisms (increased degradation and/or sequestration of the insecticide by "detoxification" enzymes) drive an important proportion of pyrethroid and DDT resistance in Africa (Martinez-Torres et al., 1998; Ranson et al., 2000). Dual inhibition of xenobiotic pumps and P450 cytochromes was also found to greatly increase *An. gambiae* susceptibility to ivermectin, suggesting a clear pathway for development of ivermectin resistance by detoxification mechanisms (Nicolas et al., 2021). Alarmingly, permethrin-resistant cockroaches, houseflies, and *Aedes aegypti* have been shown to be less susceptible to abamectin or ivermectin when compared with permethrin-sensitive counterparts (Deus et al., 2012). Given the different target of both compounds (the GluCl channels for ivermectin and the voltage-gated sodium channels for permethrin), this suggests that permethrin-ivermectin cross-resistance could occur in mosquitoes through metabolic pathways involving P450 cytochromes and/or xenobiotic pumps.

There are also some important facts to consider when studying resistance to ivermectin in mosquitoes. The susceptibility to ivermectin varies widely through different *Anopheles* species, (Ivermectin Roadmappers, 2020) and with the development stage of mosquitoes (Chen et al., 2022) (Derua et al., 2015) (Derua et al., 2016). Also, mosquito larvae are susceptible to ivermectin concentrations as low as 1 ng/ml and under (Derua et al., 2016). The potential exposure to residual ivermectin in breeding sites and how it could affect resistance must be explored by conducting targeted evolutionary experiments.

3.3. Behavioral resistance

Beside target-site mutations and enhanced metabolic clearance, behavioral changes (*i.e.* trophic avoidance) vis-a-vis to ivermectin-treated subjects could be an additional mechanism of resistance. In fact, mosquitoes have the ability to identify the vertebrate host to be bitten through a complex mixture of volatile molecules detected by their antenna (Lefèvre et al., 2010; Lehane, 2005; Marquardt, 2004). Drug metabolism has also been shown to affect the body odor of treated individuals (Zahnert et al., 2018). While a change of body odor is not reported among ivermectin's side effects, one cannot rule out the possibility of ivermectin or its metabolites to modify the odor plume of treated subjects, making them attractive or repellent to *Anopheles* mosquitoes (Fig. 3). A recent study has shown mosquitoes are capable of associating the olfactory stimulus of pesticides with their detrimental effects and subsequently avoid pesticide contact (Sougoufara et al., 2022). This mechanism could enable mosquitoes to maximize survival in environments becoming increasingly challenging owing to the intensification of chemical control interventions.

An avoidance of ivermectin-treated subjects will cause mosquitoes to systematically feed on untreated subjects such as pregnant women, children under 15 kg, those taking contraindicated drugs for co-administration (such as Ritonavir) and pregnant or milking cows in the case of cattle. In addition, if ivermectin and LLINs are both widely implemented, it is probable that mosquitoes may not be able to avoid contact with both current insecticides and ivermectin. Insecticide resistance is shown to be associated with a high fitness cost on *Anopheles* populations (delayed development of larvae, reduced survivorship of larvae and adults, reduced fecundity) (Nkahe et al., 2020; Osoro et al., 2021). Ivermectin is also known to affect mosquito survival and fecundity, and thus the ability of exposed individuals to reproduce. To survive and perpetuate the species, mosquitoes could adapt and limit their fitness loss following non-lethal exposure to insecticides and/or ivermectin as found in other insect species through an increased

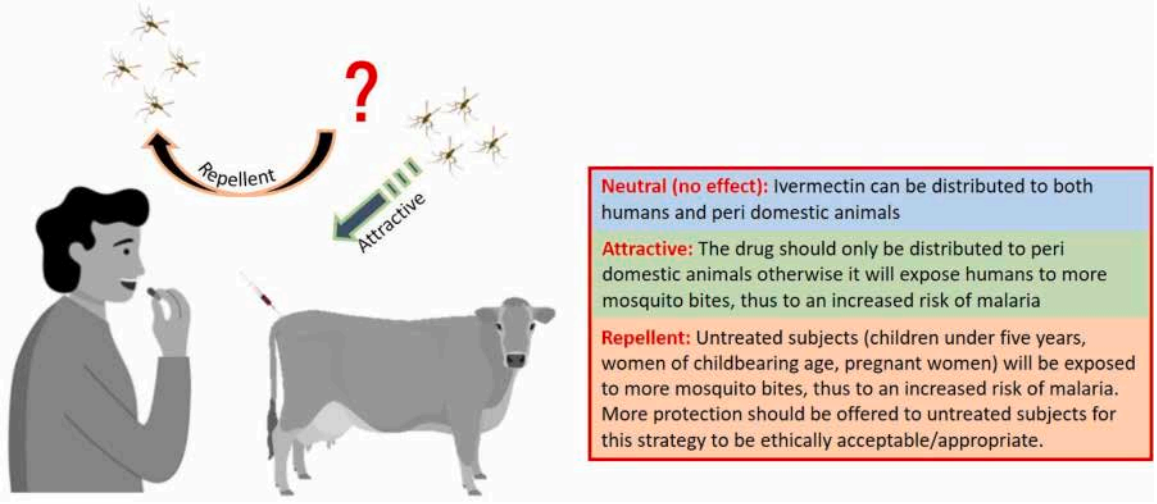

Fig. 3. Schematic representation of the attractiveness or avoidance of ivermectin-treated hosts. *The attractiveness of ivermectin-treated hosts to Anopheles mosquitoes could be studied using an olfactometer followed by a characterization of odorants using methods such as gas-chromatography coupled with mass spectrometry. These studies will determine if ivermectin-treated hosts are attractive (green arrow), repellent (orange arrow) or neural. The strategy to adopt for each scenario is described in the box.*

reproductive effort (*i.e.* adjustment of egg production) (Cutler, 2013), thermoregulation (*i.e.* rest at some specific temperatures) (Abram et al., 2017; Maliszewska and Tegowska, 2017) or self-medication (*i.e.* feeding on specific therapeutic diets like nectars) (de Roode et al., 2013; de Roode and Hunter, 2019). While these behavioral changes are yet to be observed in mosquitoes, their occurrence would greatly contribute to the spread of ivermectin resistance.

4. Research and development agenda for ivermectin resistance management

4.1. Characterization of cross-resistance to ivermectin

Several reports and models have demonstrated the importance of ivermectin MDAs in disrupting the transmission of human malaria parasites (Alout et al., 2014; Slater et al., 2020, 2014). These encouraging data in favor of a wider use of ivermectin MDAs to combat malaria need however to be analyzed more in detail by taking into consideration factors that could limit or improve its use. Among factors that could limit the use of ivermectin is the occurrence of cross-resistance between current insecticides (*i.e.* pyrethroids) and ivermectin through metabolic resistance. Several of the randomized-controlled field trials taking place are in the context of high levels of pyrethroid resistance (See Table 1). Since some pyrethroid-resistant arthropods have been shown to be less susceptible to ivermectin (Deus et al., 2012), conclusions that will be drawn from these trials should take into account potential limited effect of ivermectin on mosquito populations with some degree of metabolic resistance. The impact of pyrethroid resistance on ivermectin susceptibility in malaria vectors needs to be further explored by assessing the effects of ivermectin on adult survival and fecundity using fully susceptible and laboratory-selected permethrin resistant *Anopheles* strains for instance. As overproduction of detoxifying enzymes are one of the main mechanisms by which insects survive to insecticides, it will be critical to also characterize enzyme activity and mRNA expression levels of cytochrome P450 and glutathione S-transferase (GST) genes between ivermectin-sensitive and -insensitive pyrethroid-resistant *Anopheles* strains using both biochemical and high-throughput genome sequencing assays as performed in crop insects (Ali et al., 2019; Xu et al., 2014; Zhou et al., 2018).

The current strategy to combat metabolic-resistant malaria vectors is based on the use of LLINs combining a pyrethroid with a synergist *e.g.* piperonyl butoxide (PBO). Synergists or chemosensitizers have been found to restore the toxicity or efficacy both *in vivo* and *in vitro* when administered together with a toxicant or drug (Snoeck et al., 2017). Pyrethroid-PBO LLINs have been shown to reduce malaria prevalence in areas where they were deployed compared to pyrethroid-only LLINs (Protopopoff et al., 2018; Staedke et al., 2020). This has prompted the WHO to promote mass procurement and distribution of these LLINs to sustain vector control impact in the face of increasing metabolic resistance (PMI, 2020). Consequently, pyrethroid-PBO LLINs are rapidly replacing pyrethroid-only LLINs in many malaria endemic areas. Their proportion in Sub-Saharan Africa is on the rise going from 3% in 2018 to 51% in 2022 (AMP, 2022). This means that a deployment of ivermectin MDAs will inevitably occur in communities where pyrethroid-PBO LLINs are already or will be present. Since the PBO presents on these new generations of pyrethroid LLINs is intended to inhibit detoxification enzymes of resistant malaria vectors, mainly cytochrome P450 monooxygenases (Bingham et al., 2011), this might be beneficial for ivermectin MDAs. Indeed, the inhibition of cytochrome P450 enzymes has been shown to increase the mortality of mosquitoes when the synergist is co-administered with the blood meal (Nicolas et al., 2021). Even though mosquitoes will not be taking a blood meal containing PBO, one could expect the pre or post exposure to PBO (through pyrethroid-PBO LLINs) to synergize with ivermectin and increase mosquito mortality. Further studies will be needed to investigate the potential role of a pre or post exposure to pyrethroid-PBO LLINs in boosting ivermectin effects on mosquitoes since mosquito resistance to PBO-synergized pyrethroid has already been reported (Zhou et al., 2022).

4.2. Development of standardized methods to track ivermectin resistance

4.2.1. Molecular markers

Insecticide resistance in malaria vectors is clearly threatening current and future of vector control programs. While waiting for large-scale implementation of new vector control products, it appears essential to have a deep understanding of the mechanisms of insecticide resistance for better detection in natural mosquito populations and management. Until today, our response to this major problem has been reactive rather than proactive and understanding the molecular mechanisms involved in resistance has often been limited by available techniques. Today, advances in molecular biology offer new tools with significant potential for understanding and tracking mosquito resistance to insecticides (Faucon et al., 2017; Lol et al., 2019; Weedall et al., 2020). These advances have also opened up the potential for a more proactive approach when implementing new vector control tools such as ivermectin MDA to control malaria. Before a large-scale use of ivermectin whatever the dose, formulation and route of administration considered, it will be important to first establish ivermectin resistant phenotypes selected under several generations at the laboratory with increasing sub-lethal doses of ivermectin until resistance is observed. This could be done by either exposing larvae or adult *Anopheles* to ivermectin. Establishment of ivermectin-resistant *Anopheles* colonies is challenging because sub-lethal ivermectin concentrations can still inhibit mosquito fecundity. Finding the optimal concentration (allowing survivors to lay eggs) will be critical. If successful, the underlying mechanism(s) of the resistance can be investigated using modern high-throughput genome sequencing (RNA- and/or DNA sequencing) and big data analytics (Fig. 4). These state-off-art techniques will give the ability to predetermine alleles that are under selection and help identify potential markers of resistance (Zoh et al., 2021). Markers of ivermectin resistance could then be validated by genetic manipulation of candidate gene expression in *An. gambiae s.l.* For example, RNAi-mediated gene silencing (Mehlhorn et al., 2021) can be used to transiently suppress candidate gene expression in resistant mosquitoes prior to exposure to treated blood meals with ivermectin. The GAL4/UAS expression system in *An. gambiae s.l.* (Adolfi et al., 2019; Lynd and Lycett, 2012) can also be used to overexpress candidate genes in a susceptible mosquito population and examine resistance phenotypes through exposure to treated blood meals with ivermectin. Another promising tool for studies of gene function is the CRISPR-Cas9 system (Kistler et al., 2015). This system allows alteration of the expression of a candidate gene and examination of its impact on mosquito life traits such as development, survival, fecundity/fertility, and ivermectin resistance can be tested. Once the candidate gene or marker is validated, simplified molecular assays should be developed to track ivermectin resistance in the malaria vectors as it is currently the case for mutations associated with insecticide resistance on the voltage-gated sodium channel (VGSC) and acetylcholinesterase-1 (Ace-1) genes in malaria vectors (Lol et al., 2019; Martinez-Torres et al., 1998; Mavridis et al., 2019; Riveron et al., 2014). Such an approach before broader use of ivermectin will aid in the design of effective resistance-management strategies and establish the impact of ivermectin resistance on malaria control interventions.

4.2.2. Phenotypic assays

In addition to molecular markers to track ivermectin resistance, the development of an effective and easy-to-use phenotypic assay for resistance monitoring will provide a critical early warning in areas where ivermectin MDA is under evaluation and after large scale implementation of this strategy. Estimating the susceptibility of wild-caught adult mosquitoes to ivermectin requires they ingest ivermectin in a blood meal or in sugar water either through a membrane or direct-feeding on a treated host. Direct-feeding of mosquitoes on treated hosts

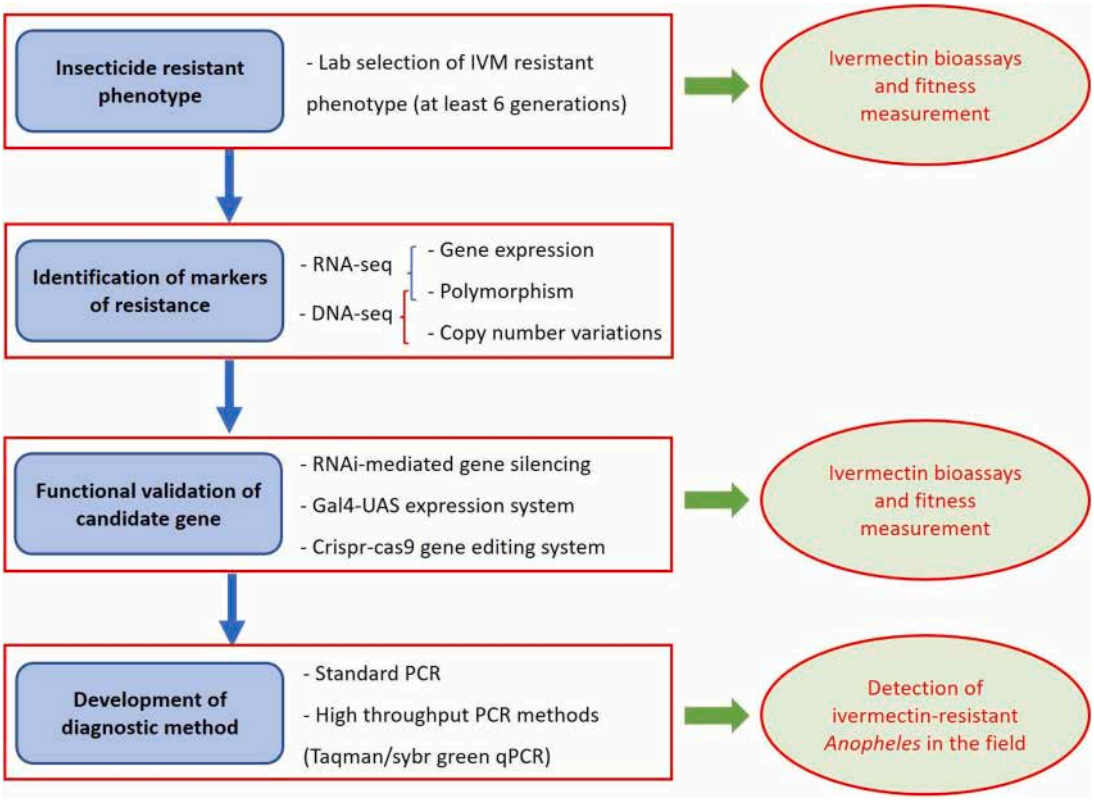

Fig. 4. Ivermectin resistance marker discovery and validation pipeline. *The discovery of resistance markers starts with evidence of decreased sensitivity against ivermectin in laboratory-selected Anopheles populations. Genomic tools could then be used to identify resistance markers by comparison of parental and selected mosquito strains albeit with the known limitations in external validity of resistant colonies generated in the insectary. Validation of a resistance candidate is often performed using multiple in vitro and in vivo strategies (RNA interference, Gal4-UAS or CRISPR-Cas9), however the choice of organism may depend upon cost, timescale, and the availability of insect culturing facilities. After the validation of the resistance candidate by ivermectin bioassays, a molecular assay will be developed and validated to monitor ivermectin resistance in the field.*

has ethical implications and membrane feeding often results in very low feeding rates. An alternative method using filter papers to deliver ivermectin in blood is currently under development with promising results (***Ominde* et al., *unpublished data***). Its use across different sites will require well-characterized standard blood or sugar-blood mixture and the development of standard operating procedures (SOPs) that will be applied to local F1 or F2 generation mosquitoes.

4.2.3. Ivermectin resistance mitigation plan

There are several endectocides available in the veterinary market. This offers the possibility to rotate or deliver a mosaic of different endectocides in herds as part of the livestock targeted intervention. Mosaics could be done within herds or by using a different endectocide in an area where humans receive ivermectin. The concept can be evaluated using ivermectin while developing novel/improved endectocides' active pharmaceutical ingredients or formulations specifically for malaria. Rotations will need at least two available endectocides for use in humans. As for now, ivermectin remains the sole endectocide (with anti-mosquito properties) approved for human use. One strategy could be the repurposing of alternative ectoparasiticidal molecules such are Isoxazolines. Isoxazolines are ligand-gated ion channel inhibitor parasiticides. They bind to gamma-aminobutyric acid (GABA)-gated chloride channels (GABACls) in nerve and muscle cells, which blocks the transmission of neuronal signals leading to death. However, their actions are putatively much more selective for GABA receptors in arthropods (fleas or ticks), than for those in mammals, including humans (Gassel et al., 2014; Shoop et al., 2014). The current registered Isoxazolines (Fluralaner and Afoxolaner) may be promising candidates for such a repurpose, given the already demonstrated mosquitocidal activity against *Anopheles* at a relatively low concentration, a much longer half-life *in vivo* (*i.e.* long time of residence) compared to ivermectin, and the commercial availability of the drugs (Miglianico et al., 2018). However, seizures and other neurotoxic effects, including death have been mentioned in some recently marketed isoxazoline package inserts (Palmieri et al., 2020). It would therefore require an extensive work package to get an approval human use. Further studies will be needed to calculate the optimal design of rotational treatment in livestock or human that prevents the development and spread of ivermectin resistance in malaria vectors.

Another approach could be the use of ivermectin in human or livestock in combination with a synergist. This strategy is currently used to combat pyrethroid-resistant malaria vectors by combining pyrethroids and PBO on LLINs (Accrombessi et al., 2021; Protopopoff et al., 2018). Cyclosporin A and Verapamil (ABC-transporter inhibitors) have shown to cause an increase in the toxicity against ivermectin in cattle tick, *Rhipicephalus microplus* (Pohl et al., 2014; P.C. 2012, 2011) and in *Culex pipiens* mosquitoes (Buss et al., 2002). Similarly, Voriconazole (Dual CYP/P-gp inhibitor) has a synergistic effect on ivermectin-induced *An. gambiae* mortality (Nicolas et al., 2021). In the case of ivermectin, some synergist may simultaneously work as pharmaco-enhancers, boosting both the systemic exposure and the mosquito susceptibility (Chaccour et al., 2017). The use of synergists to increase the toxicity of ivermectin in mosquito populations could avoid further selection of resistant malaria vectors and thereby delays the spread of ivermectin resistance. Although all these drugs used as synergists have already been approved for human use, their combination with ivermectin in livestock and humans will require extensive studies on their pharmacokinetics and safety given their potential effect on the blood-brain barrier.

Additionally, attention must be paid to other potential uses that can increase the exposure and hasten the appearance of resistance such as

ivermectin-based sugar baits (Tenywa et al., 2017) or wall linings (Malima et al., 2017).

5. Concluding remarks

Although there are numerous laboratory and field studies demonstrating the efficacy of ivermectin for controlling malaria vectors, data from randomized-controlled field trials demonstrating a direct impact of this strategy on malaria transmission are still awaited. So far, only two published studies have examined the efficacy of repeated MDAs of ivermectin on the incidence of malaria in a two-arm, cluster-randomized controlled trial (Dabira et al., 2022; Foy et al., 2019). However, these studies did not demonstrate a clear effect of ivermectin on malaria prevalence or transmission. Preliminary data of the RIMDAMAL II project also showed no effect of ivermectin when added to current malaria strategies (***Foy* et al.*, unpublished data***). Several other trials are still ongoing and will show us in years to come whether MDAs of ivermectin are effective or not for controlling malaria.

In the meantime, it is critical to anticipate the research on ivermectin resistance in mosquitoes (**see pending questions**) and identify effective resistance-management strategies in case this strategy is evidenced as efficacious in controlling malaria. Molecular markers of ivermectin resistance need to be identified and developed into validated assays as well as susceptibility tests (phenotypic assays). However, the susceptibility of mosquitoes to ivermectin is species-dependent, varying from 15.9 ng/ml (7-day LC_{50}) in *An. gambiae* (Smit et al., 2018) to much higher, 55.6 ng/ml (7-day LC_{50}) for *An. dirus* (Kobylinski et al., 2017). Efforts to characterize major malaria vectors in different geographic areas should be done prior to using ivermectin for vector control. In addition, susceptibility tests should be validated from both colony and wild-type mosquitoes to determine the dose-defining species, the dose that is needed to kill the less susceptible malaria vector. This will not be achieved without first standardizing outcome metrics such as lethal concentration 50, since a variety of time intervals are often presented varying from 24 h to over 10 days.

6. Pending questions

- Ivermectin is delivered to the gut via the blood meal, how does this affect potential resistance mechanisms? *i.e.* cuticular efflux pumps and pre-systemic metabolism
- How does the glutamate-gated chlorine channel expression vary across different mosquito development stages and species?
- What is the window of selection of ivermectin and how does it vary across *Anopheles* species?
- What is the most likely ivermectin resistance mechanism in mosquitoes?
- How will ivermectin resistance occur (and how fast) during or after the numerous ivermectin trials in malaria vectors?
- What is the best way to monitoring ivermectin for resistance (SNPs, gene mutations, phenotype assays) during trials?
- What is the ideal strategy combination to assess/prevent ivermectin resistance in mosquitoes?

Glossary

Anthropophilic: Describes mosquitoes that prefer to take blood meals from humans

Dose-defining species: For antiparasitic drugs with a broad spectrum of activity like ivermectin, the dose-defining species refers to the dose defined to target the parasite (herein an *Anopheles* species) that requires the highest drug exposure to be suppressed.

Endectocide: A drug with a wide spectrum of activity capable of killing both endoparasites (intestinal nematodes) and ectoparasites (blood-sucking insects)

Endophagic: An endophagic mosquito is a mosquito that feeds indoors, inside human habitats

Endophilic: An endophilic mosquito is a mosquito that tends to inhabit/rest indoors

Exophagic: An exophagic mosquito is a mosquito that feeds outdoors.

Exophilic: An exophilic mosquito tends to inhabit/rest outdoors.

Residual malaria transmission: Residual malaria transmission is the fraction of total transmission that persists after achievement of full operational coverage with effective long-lasting insecticidal nets and/or indoor residual spray interventions.

Rotation: If molecules with the same mode of action are used repeatedly, this will result in increased selection pressure on an arthropod pest population, which enhances the rate (speed) of resistance development. Endectocide rotation is the temporal (time) alternation of endectocides with different modes of action to slow down the development of resistance.

Self-medication: the ability of insects to consume or otherwise contact biologically active organic compounds specifically for the purpose of helping to reduce the deterrent effects of insecticides.

Synergist: A chemical that enhances the effectiveness of an active agent

Thermoregulation: also known as temperature regulation, describes the ability of insects and other animals to maintain a stable temperature (either above or below ambient temperature), at least in a portion of their bodies by physiological or behavioral means.

Window of escalation: when the dose gap between the dose-defining species and the more susceptible ones is large (*e.g.* for ivermectin 20 vs 200 mcg/kg), resistance arising in the most susceptible organisms cannot be clinically detected until the intensity has risen to the level of the highest dose (10-fold for ivermectin).

Window of selection: refers to time period during which the drug exposure is low enough to allow mutant organisms to reproduce but high enough to suppress the development of wild-type ones. This allows for amplification of the mutant phenotype.

Zoophagic: Describes mosquitoes that prefer to take blood meals from animals

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CRediT authorship contribution statement

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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