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Natural occurrence of *Wolbachia* in *Anopheles* sp. and *Aedes aegypti* populations could compromise the success of vector control strategies

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Wolbachia is a maternally inherited bacterium commonly detected in approximately 50% of arthropod species, including mosquito vector species. *Wolbachia* species have been detected in different mosquito vectors, but in most malaria vectors, their occurrence in natural populations were reported 10 years ago. *Aedes aegypti*, the main vector of dengue virus, is generally uninfected by *Wolbachia*, and records of infection are rare and only include a few populations. This bacterium impacts the biology, ecology, and evolution of vector populations. *Wolbachia* has attracted considerable interest because of its role in reducing disease transmission. Moreover, this bacterium is known to manipulate insect reproduction by inducing cytoplasmic incompatibility (CI), thus providing new avenues for vector control strategies. Interestingly, *wMel* or *wAlbB Wolbachia* infections in *Aedes* populations exhibit a stable high frequency in most areas and contribute to the reduction of local dengue transmission. In natural populations of *Anopheles*, although *Wolbachia* was found, little is known about its role and effect on *Plasmodium*. If the incompatible insect technique (IIT) and population replacement strategy resulted in significant decreases in the dengue transmission in endemic countries such as the USA, Taiwan, Australia, and Brazil, natural *Wolbachia* detection in mosquitoes may pose a threat to these vector control strategies, raising the following question: "Does the natural occurrence of *Wolbachia* in *Anopheles* sp. and *Ae. aegypti* populations compromise the success of vector control strategies? This review presents recent achievements of *Wolbachia* in natural *Anopheles* and *Ae. aegypti* populations in terms of prevalence and provides guidelines for the development of *Wolbachia*-based vector control.

KEYWORDS

***Wolbachia*, *Aedes aegypti*, *Anopheles* sp. prevalence, cytoplasmic incompatibility, vector control**

1 Introduction

In tropical and subtropical regions, dengue and malaria remain the two main vector-borne infectious diseases transmitted by *Aedes aegypti* and *Anopheles gambiae* s.l., respectively.

Malaria is a life-threatening disease caused by parasites transmitted to people through the bites of infected female *An. gambiae* s.l mosquitoes. Overall, each year, the number of infected people varies from 154 to 289 million, with approximately 80% of all malaria deaths recorded mostly in children under 5 years of age in endemic regions of Africa (1). Among the arboviroses, i.e., yellow fever virus (YFV), Zika virus, Chikungunya virus (CHYKV), and dengue virus, transmitted generally by *Ae. aegypti*, dengue virus is the most prevalent in subtropical and tropical areas and remains a major public health concern (2).

Vector control based on chemicals remains the most effective strategy for controlling the transmission of dengue and malaria diseases. Long-lasting insecticide-treated nets (LLINs) and indoor residual spraying (IRS) are the main vector control strategies (3). These methods have significantly contributed to a decrease in malaria incidence. However, the effectiveness of vector control may be constrained by the increasing insecticide resistance in *Anopheles* vectors in many countries, which has now been observed in almost all African countries (4). Cases of insecticide resistance in *Aedes* populations have also been reported in many areas (5).

With regard to insecticide resistance occurring in several areas of endemic countries, particularly for both diseases, there is a need for new vector control technologies. Existing control methods, including environmental/mechanical (e.g., reduction source or destruction of breeding sites), biological (e.g., *Bacillus thuringiensis* var. *israelensis*, entomopathogenic fungi, larvivorous fish, and copepods), chemical (e.g., insect growth regulators, pyrethroids, and DDT), and endosymbiont *Wolbachia* and genetic methods (e.g., sterile insect technique and genetically modified mosquitoes), can contribute to a decrease in dengue vector populations (5, 6) and in malaria vectors.

Innovative eco-friendly approaches for the control of vector diseases are under active development and could complement the current mosquito control strategies (5). Among the most promising techniques, the use of essential oils (7–10) has provided valuable data in terms of alternative vector control. The sterile insect technique (SIT; i.e., the use of males sterilized by irradiation) and the incompatible insect technique (IIT; which uses *Wolbachia* endosymbionts to induce cytoplasmic incompatibility) could lead to population suppression, and the release of males reduces the fertility of wild females (11).

Wolbachia is an endosymbiotic, Gram-negative intracellular bacterium described for the first time within the reproductive tissues of *Culex pipiens* mosquitoes in 1924 (12). Most of the arthropod *Wolbachia* strains belong to clades A and B, whereas clades C and D are observed in filarial nematodes (13). These bacteria, which are members of the order Rickettsiales within the class α-Proteobacteria, cannot be cultured outside the host cells. According to Weinert et al. (14) and Bailly-Bechet et al. (15), *Wolbachia* is considered to be the most abundant symbiont and has been found to infect approximately 50% of all arthropod species.

In previous decades, *Wolbachia* has received special attention due to the diversity of its phenotypes, including reproductive manipulations (16–19), nutrient synthesis (20), physiological and behavioral modifications, and its impacts on susceptibility to pathogens (21–25).

To the best of our knowledge, studies have reported the occurrence of *Wolbachia* in around 31 species of *Anopheles*, but a few of these studies did not investigate its ability to inhibit *Plasmodium* in host populations. Moreover, a lower prevalence (from 0.2% to 13.24%) of *Wolbachia* was found in *Ae. aegypti* populations from Manila (Philippines), Florida, and Panama, whereas those found in New Mexico reached 57% (26). Thereafter, a number of *Ae. aegypti* samples from New Mexico were screened for confirmation (27). Thus, both real-time PCR and loop-mediated isothermal amplification (LAMP) assays were performed, but no *Wolbachia wAlbB* strain infection was detected among 120 individual mosquitoes that previously tested positive for *Wolbachia* (27). According to these studies, molecular detection methods (e.g., LAMP, PCR, antibiotic treatment, intracellular localization by STEM, and FISH) are useful for confirming the presence of *Wolbachia*.

The two main *Wolbachia*-based strategies for the reduction of disease transmission are IIT or population suppression and population replacement (see Figure 1). Their implementation through the consecutive releases of males artificially infected with *Wolbachia*-inducing cytoplasmic incompatibility (CI) and female populations involved in *Wolbachia* infection transmission have at times been proven to reduce vector competence (28).

This review presents recent insights into *Wolbachia* in natural *Anopheles* sp. and *Ae. aegypti* populations in terms of prevalence and outlines its major role in pathogen transmission. It also focuses on the implications of natural infections in *Anopheles* and *Aedes* populations for *Wolbachia*-based disease control strategies.

2 What are endosymbiotic *Wolbachia*?

Wolbachia is naturally found in many species of arthropods, but can also be transfected to prevent the transmission of diseases (12). This bacterium belongs to the α-Proteobacteria within the order Rickettsiales. It cannot be cultivated outside the host cells. Based on genetic similarity, *Wolbachia* species are divided into supergroups A, B, C, D, E, F, and H, which appear to be linked to particular host classes (29). Recently, a novel supergroup named S has been identified in the pseudoscorpion *Cordylochernes scorpioides*, which is most closely related to *Wolbachia* supergroups C and F (30).

Wolbachia species are vertically and maternally transmitted through the egg cytoplasm and manipulate host reproduction by inducing CI (Figure 2), feminization, killing of male embryos, and parthenogenesis to enhance their spread (12, 29). Maternal

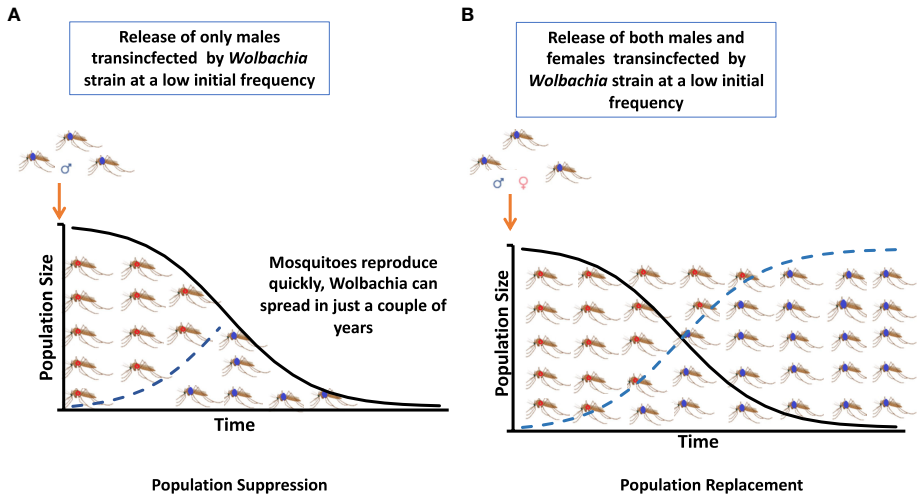

FIGURE 1
Two strategies based on *Wolbachia* for the reduction of disease transmission are an incompatible insect technique (IIT) or population suppression and a population replacement strategy. **(A)** Population suppression. **(B)** Population replacement.

transmission and the induction of various phenotypes in the hosts remain the two key features induced by this bacterium (31).

In CI (see Figure 2), *Wolbachia* induces the death of embryonic offspring from a cross between uninfected females and infected males (18). As far as male killing is concerned, this bacterium causes the death of male offspring (18, 31). Finally, parthenogenesis and feminization induction result from the transformation of potential males into females. In parthenogenesis, zygotes can develop without mating (18, 31).

In general, all phenotypes increase the number of infected females in the host population, thereby increasing the transmission of endosymbionts to the next generation. According to LePage and Bordenstein (29), when a *Wolbachia* strain (e.g., that induced CI) infection is viable, this leads to a fitness advantage of

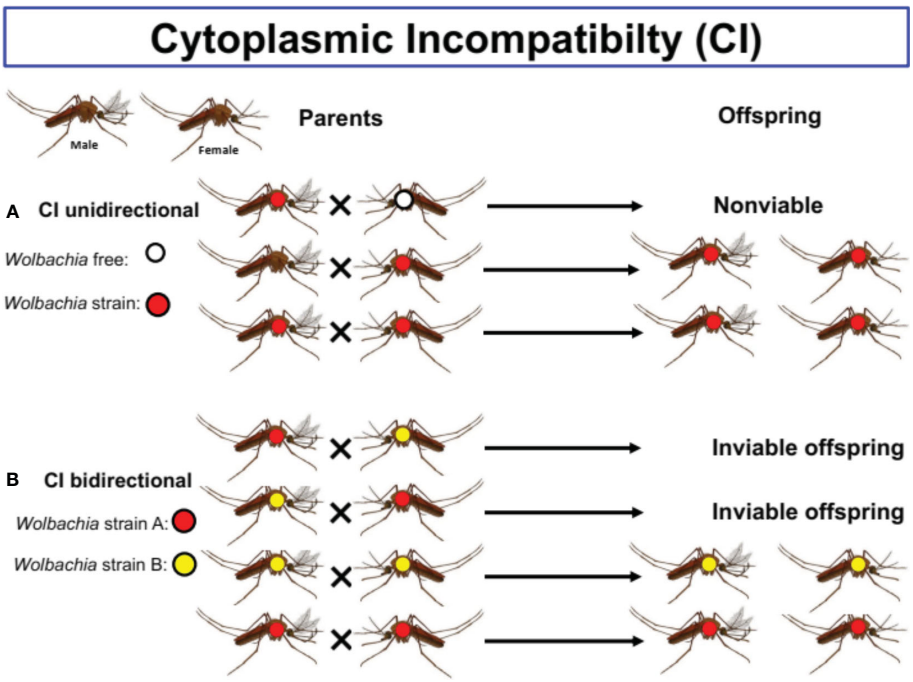

FIGURE 2
The different types Cytoplasmic Incompatibility (CI) in mosquitoes species. Cytoplasmic incompatibility (CI) prevents infected males from successfully mating with females that lack the same Wolbachia types. **(A)** Unidirectional CI occurring between Wolbachia infected males and natural uninfected females allows Wolbachia to invade uninfected populations. **(B)** Bidirectional CI occurring when both reciprocal crosses between males and females infected with different strains of Wolbachia are incompable.

the infected females compared with uninfected females. The discovery of the *Wolbachia* strains *wMel*, showing the capacity to reduce vector competence (32); *wMelpop*, favoring life-shortening of infected individual populations; and *wAlB*, highlighting increased resistance against infectious agents causing diseases, provides promise in terms of vector control strategies (12, 29). With these different phenotypes, *Wolbachia* can be used as a control agent against vector-borne diseases (22, 27, 33).

3 *Wolbachia* and *Anopheles* vector populations: prevalence, co-occurrence *of Wolbachia* and *Plasmodium*, and effect on *Plasmodium* sp.

3.1 Prevalence of *Wolbachia* in natural *Anopheles* vector populations

Although *Wolbachia* has been found in approximately 40% of 147 culicine species such as *Culex* sp. and *Aedes albopictus*, it was not detected in *Anopheles* mosquitoes until its first occurrence in natural populations in Africa approximately 10 years ago (34).

According to Bourtzis et al. (35), the absence of *Wolbachia* could be the outcome of incompatible physiological environments in *Anopheles* mosquitoes, an inability to obtain *Wolbachia* by horizontal transmission from other species, or a putative competitive exclusion by native bacteria in *Anopheles* spp.

In general, nested PCR-targeted 16S rRNA sequencing, quantitative PCR (qPCR), and electron microscopy are used for the detection of *Wolbachia* in *Anopheles* populations in most studies. Table 1 displays the techniques used by Walker et al. (36) combining molecular detection and electronic microscopy for the detection of *Wolbachia*.

Therefore, to show evidence of a stable, maternally transmitted *Wolbachia* in a host (45), a number of steps must be highlighted, as follows: i) examining *Wolbachia* in different host tissues using fluorescence *in situ* hybridization (FISH) or electron microscopy; ii) exhibiting that *Wolbachia* is and can be maternally transmitted following reciprocal crosses; and iii) showing that this bacterium can be blocked in mosquitoes with antibiotic treatment (27).

The prevalence of *Wolbachia* in *Anopheles* mosquitoes could be influenced by either i) native microbiota interference or ii) *Wolbachia*–host interaction. *Wolbachia* coexists with native microbiota that could interfere with other bacteria, leading to a lower prevalence rate. Evidence suggests that *Asaia*, a native microbiome in *Anopheles* mosquitoes, impedes the vertical transmission of *Wolbachia* (46, 47) and represents an eventual competitor to *Wolbachia*. *Asaia* inhibits the maternal transmission of Wolbachia (48) and could induce the mutual exclusion of *Wolbachia* in the gonads (49).

Globally, the mechanisms that limit the levels of *Wolbachia* are not well elucidated. One hypothesis is that *Wolbachia* can adapt to replication control as a strategy to evade host immunity (50). It is also possible that *Anopheles* does not represent a suitable host for *Wolbachia*. Moreover, the immunity or metabolism of *Anopheles* might limit the presence of *Wolbachia*.

In *Aedes*, *Wolbachia* manipulates the metabolism of host lipids, and cholesterol sequestration might favor protection against viruses (51). The lipid metabolism in *Anopheles* is not well known; however, poor nutritional stores could explain the inability of *Anopheles* mosquitoes to support the high densities of *Wolbachia* (50).

For a long time, it was believed that *Wolbachi*a is absent in wild *Anopheles* mosquitoes, until 2014, when the *WAnga*-Burkina Faso (*wAnga*-BF) strain was detected in *An. gambiae* collected from Burkina Faso, West Africa (34). This strain was different from those infecting other arthropods, including mosquitoes and other insects (52, 53).

A few years later, other findings showed the occurrence of *Wolbachia* in other *Anopheles* vectors in Africa (36, 37, 39–41, 43) and Southeast Asia (Myanmar and India) (54) (Table 1). The bacterium was found not only in *An. gambiae* and *Anopheles coluzzii* but also in other *Anopheles* species such as *Anopheles arabiensis* (37; 42); *Anopheles demeilloni* (36); *Anopheles moucheti* (36, 43); *Anopheles funestus* (40); *Anopheles melas* (55); *Anopheles nili* and *Anopheles coustani* (43); *Anopheles maculatus* (s.s.), *Anopheles sawadwongporni*, *Anopheles pseudowillmori*, *Anopheles dirus* (s.s.), and *Anopheles baimaii* (38); *Anopheles carnevalei*, *Anopheles hancocki*, *Anopheles implexus*, *Anopheles jebudensis*, *Anopheles marshallii*, *Anopheles nigeriensis*, *Anopheles paludis*, and *Anopheles vinckei* (43); *Anopheles balabacensis*, *Anopheles latens*, *Anopheles introlatus*, *Anopheles macarthuri*, *Anopheles barbirostris*, *Anopheles hyrcanus*, and *Anopheles sinensis* (44); and *Anopheles culicifacies* and *Anopheles stephensi* (54), totaling around 31 species of *Anopheles* harboring *Wolbachia* (Table 1).

To date, the prevalence of *Wolbachia* has been documented in around 20 wild *Anopheles* mosquito species. This prevalence varied according to both the location and the *Anopheles* species. A prevalence of 1% was found in *An. funestus* in Senegal, West Africa (40), and in *Anopheles minimus*, *An. dirus*, *An. maculatus*, *An. pseudowillmori*, *Anopheles baimii*, and *An. sawadwongporni* in Kayin state (38). Prevalence rates ranging from 2% to 15% were recorded in *An. culicifacies* and *An. stephensi* from India (54); in *An. carnevalei*, *An. hancocki*, *An. implexus*, *An. marshallii*, *An. nigeriensis*, *An. paludis*, and *An. vinckei* populations from Gabon, Central Africa (43); in *An. arabiensis* from Tanzania (37); and in *An. melas* from Guinea (55).

The lower prevalence rate of *Wolbachia* in *Anopheles* mosquitoes raises many questions, which could be due to the absence of a stable relationship between *Wolbachia* and its hosts. According to Chrostek and Gerth (45), the detection of *Wolbachia* in the *An. gambiae* population was surprising, even though its maternal transmission has been proven in the natural population of *An. gambiae* s.l. (34, 41, 56). The authors believed that the occurrence of *Wolbachia* is the result of contamination through several sources and could have been transferred firstly via endoparasitic nematodes or ectoparasitic mites, secondly via plants when *Wolbachia* might have been transferred from infected to uninfected insects found on the same plants, and thirdly via the water bodies of cohabitating insects infected by

TABLE 1 Prevalence of *Wolbachia* in natural *Anopheles* vector populations.

Wolbachia strain	Supergroup	Mosquito species	Type of collection	Detection technique	Individuals tested (n)	Prevalence (%)	Collection site	Country	Collection year	Reference
wAnM	B	*An. moucheti*	Outdoor	16S rRNA, sequencing, qPCR, MLST, and electron microscopy	1,086	56.6	Cameroon	Cameroon	2015	Walker et al. (36)
wAnD	B	*An. demeilloni*	Outdoor	16S rRNA, sequencing, qPCR, MLST, and electron microscopy	302	38.7	Kenya	Kenya	2011–2012	Walker et al. (36)
wAnD	B	*An. demeilloni*	Outdoor	16S rRNA, sequencing, qPCR, MLST, and electron microscopy	178	89.3	DRC	DRC	2015	Walker et al. (36)
wAnD	B	*An. demeilloni*	Outdoor	16S rRNA, sequencing, qPCR, MLST, and electron microscopy	8	100.00	DRC	DRC	2019	Walker et al. (36)
wAnga_TZ	B	*An. arabiensis*	Outdoor	16S rRNA, sequencing	65	3.1	Lupiro	Tanzania	2014	Baldini et al. (37)
wAnga_TZ	B	*An. arabiensis*	Outdoor	16S rRNA, sequencing	147	7.5	Lupiro	Tanzania	2016	Baldini et al. (37)
NA	F/D	*An. minimus*	Outdoor	16S rRNA sequencing, qPCR	90	0.033	Kayin state	Myanmar	2017	Sawasdichai et al. (38)
NA	B	*An. dirus*	Outdoor	16S rRNA sequencing, qPCR	12	0.08	Kayin state	Myanmar	2017	Sawasdichai et al. (38)
NA	B/F	*An. maculatus*	Outdoor	16S rRNA sequencing, qPCR	90	0.04	Kayin state	Myanmar	2017	Sawasdichai et al. (38)
NA	B	*An. pseudowillmori*	Outdoor	16S rRNA sequencing, qPCR	11	0.09	Kayin state	Myanmar	2017	Sawasdichai et al. (38)
NA	B/D	*An. baimii*	Outdoor	16S rRNA sequencing, qPCR	93	0.02	Kayin state	Myanmar	2017	Sawasdichai et al. (38)
NA	B	*An. sawadwongporni*	Outdoor	16S rRNA sequencing, qPCR	68	0.01	Kayin state	Myanmar	2017	Sawasdichai et al. (38)
NA	A	*An. stephensi*	Outdoor	16S rRNA sequencing and MLST	46	15.2	Godey	Ethiopia	2018	Waymire et al. (39)
NA	A	*An. stephensi*	Outdoor	16S rRNA sequencing and MLST	46	15.2	Semera	Ethiopia	2018	Waymire et al. (39)
NA	A?	*An. stephensi*	Outdoor	16S rRNA sequencing and MLST	50	4	Dire Dawa	Ethiopia	2018	Waymire et al. (39)
NA	A	*An. stephensi*	Outdoor	16S rRNA sequencing and MLST	24	9.1	Kebridehar	Ethiopia	2018	Waymire et al. (39)

(Continued)

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TABLE 1 Continued

Wolbachia strain	Supergroup	Mosquito species	Type of collection	Detection technique	Individuals tested (n)	Prevalence (%)	Collection site	Country	Collection year	Reference
wAnfu-Senegal	A	*An. funestus*	Outdoor	16S rRNA sequencing and qPCR	247	1.2	Dielmo	Senegal	2014	Niang et al. (40)
NA	A/B	*An. coluzzii*	Mating swarms	16S rRNA sequencing and qPCR	36	19.44	VK5	Burkina Faso	2011	Baldini et al. (34)
NA	A/B	*An. coluzzii*	Mating swarms	16S rRNA sequencing and qPCR	3	7.1	VK3	Burkina Faso	2011	Baldini et al. (34)
*wAnga*_Burkina	A/B	*An. gambiae*	Mating swarms	16S rRNA sequencing and qPCR	24	4.2	Soumousso	Burkina Faso	2011	Baldini et al. (34)
*wAnga*_Mali	A/B	*An. gambiae* s. l.	Indoor collection	16S rRNA sequencing, qPCR, and MLST	25	76	Kenieroba	Mali	2010	Gomes et al. (41)
*wAnga*_Mali	A/B	*An. gambiae* s. l.	Indoor	16S rRNA sequencing, qPCR, and MLST	83	78	Kenieroba	Mali	2015	Gomes et al. (41)
*wAnga*_Mali	A/B	*An. gambiae* s. l.	Indoor	16S rRNA sequencing, qPCR, and MLST	44	61	Dangassa	Mali	2010	Gomes et al. (41)
*wAnga*_Mali	A/B	*An. gambiae* s. l.	Indoor	16S rRNA sequencing, qPCR, and MLST	116	46	Dangassa	Mali	2015	Gomes et al. (41)
*wAnga*_Burkina	B	*An. coluzzii*	outdoor	16S rRNA and *wsp* gene sequencing and MLST	287	4.2	Dogo	Ghana	2013–2017	Jeffries et al. (42)
NA	B	*An. gambiae* s.s.	outdoor	16S rRNA and *wsp* gene sequencing and MLST	36	7.7	Kinshasa	DRC	2013–2017	Jeffries et al. (42)
NA	B	*An. minimus*	Outdoor	16S rRNA sequencing and MLST	NA	NA	Gabon	Gabon	2012–2016	Ayala et al. (43)
NA	B	*An. nigeriensis*	Outdoor	16S rRNA sequencing and MLST	27	4	Gabon	Gabon	2012–2016	Ayala et al. (43)
NA	B	*An. paludis*	Outdoor	16S rRNA sequencing and MLST	16	6	Gabon	Gabon	2012–2016	Ayala et al. (43)
NA	A/B	*An. vinckei*	Outdoor	16S rRNA sequencing and MLST	30	10	Gabon	Gabon	2012–2016	Ayala et al. (43)
NA	B	*An. balabacensis*	Outdoor	16S rRNA sequencing and MLST	19	21.1	Gabon	Gabon	2012–2016	Ayala et al. (43)
NA	A/B	*An. introlatus*	Outdoor	16S rRNA and *wsp* gene sequencing	NA	16.7	Ulu Kalong, Selangor forest	Malaysia	2013–2019	Wong et al. (44)

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TABLE 1 Continued

Wolbachia strain	Supergroup	Mosquito species	Type of collection	Detection technique	Individuals tested (n)	Prevalence (%)	Collection site	Country	Collection year	Reference
NA	B	*An. maculatus*	Outdoor	16S rRNA and *wsp* gene sequencing	NA	100	Ulu Kalong, Selangor forest	Malaysia	2013–2019	Wong et al. (44)
NA	B	*An. barbirostris*	Outdoor	16S rRNA and *wsp* gene sequencing	NA	20	Putrajaya wetland	Malaysia	2013–2019	Wong et al. (44)
NA	A/B	*An. hyrcanus*	Outdoor	16S rRNA and *wsp* gene sequencing	NA	40	Putrajaya wetland	Malaysia	2013–2019	Wong et al. (44)
NA	A/B	*An. hyrcanus*	Outdoor	16S rRNA and *wsp* gene sequencing	NA	75	Bukit Lagong, Selangor forest	Malaysia	2013–2019	Wong et al. (44)
NA	A/B	*An. hyrcanus*	Outdoor	16S rRNA and *wsp* gene sequencing	NA	50	Sg. Sendat, Selangor forest	Malaysia	2013–2019	Wong et al. (44)
NA	B	*An. sinensis*	Outdoor	16S rRNA and *wsp* gene sequencing	7	57.1	Sg. Sendat, Selangor forest	Malaysia	2013–2019	Wong et al. (44)
NA	NA	*An. macarthuri*	Outdoor	16S rRNA and *wsp* gene sequencing	NA	25	Tawau, Sabah forest	Malaysia	2013–2019	Wong et al. (44)
NA	A	*An. latens*	Outdoor	16S rRNA and *wsp* gene sequencing	NA	20	Tawau, Sabah forest	Malaysia	2013–2019	Wong et al. (44)
NA	B	*An. balabacensis*	Outdoor	16S rRNA and *wsp* gene sequencing	NA	23.5	Tawau, Sabah forest	Malaysia	2013–2019	Wong et al. (44)
NA	B	*An. barbirostris*	Outdoor	16S rRNA and *wsp* gene sequencing	NA	100	Tawau, Sabah forest	Malaysia	2013–2019	Wong et al. (44)
NA	A/B	*An. introlatus*	Outdoor	16S rRNA and *wsp* gene sequencing	NA	55.6	Kluang, Johor forest	Malaysia	2013–2019	Wong et al. (44)
NA	A/B	*An. introlatus*	Outdoor	16S rRNA and *wsp* gene sequencing	NA	24.2	Mersing, Johor forest	Malaysia	2013–2019	Wong et al. (44)
NA	A/B	*An. latens*	Outdoor	16S rRNA and *wsp* gene sequencing	NA	100	Mersing, Johor forest	Malaysia	2013–2019	Wong et al. (44)
NA	A/B	*An. introlatus*	Outdoor	16S rRNA and *wsp* gene sequencing	NA	50	Kota Tinggi, Johor forest	Malaysia	2013–2019	Wong et al. (44)
NA	A/B	*An. latens*	Outdoor	16S rRNA and *wsp* gene sequencing	NA	50	Kota Tinggi, Johor forest	Malaysia	2013–2019	Wong et al. (44)

Molecular detection was performed using 16S rRNA sequencing, wsp (Wolbachia surface protein) gene sequencing, qPCR (a quantitative PCR-based detection method performed to establish both the prevalence and the intensity of Wolbachia infection), MLST (multilocus sequence typing), and electron microscopy using fluorescence in situ hybridization (FISH).
NA, not applicable; DRC, Democratic Republic of the Congo.

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Wolbachia. According to Chrostek and Gerth (45), the high diversity of *Wolbachia* sequences associated with very low titers was incompatible with the notion of a stable, intraovarially transmitted *Wolbachia* symbiont in *An. gambiae*.

A proportion of the population of *Anopheles* displayed a prevalence rate ranging from 20% to 60%. This was the case for *An. gambiae* collected indoors in Mali (41); *An. gambiae* s.s. from Kalemie in the DRC (57); *An. gambiae* s.s.–*melas* hybrids from Guinea (55); *An. nili* collected in Gabon (43); and *An. sinensis*, *An. introlatus*, and *An. latens* collected in forest areas (44) (Table 1).

Interestingly, higher prevalence rates were found in *An. demeilloni* collected in the DRC, Central Africa (89.3% and 100% in 2015 and 2019, respectively) (36); in *An. moucheti* (71%) (43) and *An. gambiae* (78%) in Mali (57) (41); and in *An. maculatus*, *An. barbirostris*, and *An. latens*, with prevalence reaching 100% (44), suggesting that natural *Wolbachia* infections are widespread in these species of *Anopheles*. Walker et al. (36) provided evidence that *An. demeilloni* and *An. moucheti* harbor a high density of *Wolbachia* strains acquired vertically. Using phylogeographic sequencing data (*wsp* gene and MLST sequences), the authors showed that the *wAnM* strain from *An. moucheti* and the *wAnD* strain from *An. demeilloni* span wide geographical locations, which is consistent with the notion of a stably inherited CI induced by *Wolbachia* strains (43). Thus, the prevalence rates in wild mosquito populations are also consistent with CI-inducing strains, in contrast to most studies exhibiting a low prevalence of *Wolbachia* in the *An. gambiae* complex. Interestingly, sequencing of the *wAnM* genome revealed an interrupted *cifB* gene that could also be indicative of a variation in the levels of CI induced by this strain (43). Through experiments conducted by Adams et al. (58), it was shown that *Wolbachia cifB* induced CI in *An. gambiae* individuals and that the *cifB*-induced sterility was rescued by the expression of *cifA* in females. According to Ayala et al. (43), analysis of *An. moucheti* from an F1 progeny confirmed the absence of biological *Wolbachia* contamination in their studies. They also suggested that *Wolbachia* is maternally inherited in wild populations of *An. moucheti*. Therefore, it should be considered as a potential model species for further investigations of its interactions with *Plasmodium* infections.

According to Wong et al. (44), vegetation influences the prevalence of *Wolbachia*, which can be higher in forested areas than in wetlands or islands. The authors believed that the diversity and abundance of the flora and fauna in forested areas harboring more hosts with stable *Wolbachia* might favor horizontal transfers to other species.

For Hemingway et al. (59), the widespread insecticide resistance observed in malaria vectors in Africa could also explain the spread of *Wolbachia* into *Anopheles* populations, resulting in a reduction of malaria transmission, which is in contrast with that observed in Burkina Faso. *Wolbachia* was found in *An. gambiae* s.s. in 2006 in Soumousso and VK7 (60), during which the frequency of resistance in these populations was low. After 12 years (2018), coinciding with the high levels of insecticide resistance, *Wolbachia* was not detected in mosquito populations, raising doubts about the persistence of this bacterium under insecticide pressure. In the mosquito *C. pipiens*, the physiological costs associated with insecticide resistance limit the ability to control *Wolbachia* infection (61, 62). Additional investigations under different ecological settings and mosquito host genetic backgrounds are therefore needed to understand the factors affecting the dynamics of *wAnga* infection and the role of *wAnga* in vectorial capacity.

3.2 Co-occurrence of *Wolbachia* and *Plasmodium* in natural *Anopheles* and their interactions

In mosquitoes, oocyst development can be perturbed when *Wolbachia* is naturally found in the mosquitoes (63). Conversely, it can favor sporozoite production (64). More sporozoites were detected in *C. pipiens*, a vector of *Plasmodium relictum* naturally infected by *Wolbachia*, compared with those in *C. pipiens* without *Wolbachia* (65).

Interestingly, quantitative analysis of *Wolbachia* in *wAnga*-Mali and *Plasmodium* sporozoite infections in natural *An. gambiae* s.s. populations from Mali, West Africa, indicated a lower prevalence and intensity of *Plasmodium falciparum* sporozoite infection in *Wolbachia*-infected females (41). The presence of *wAnga*-BF was negatively correlated with the prevalence of *P. falciparum* sporozoites (56). According to Wong et al. (44), *Anopheles* mosquitoes with sporozoites (50%) exhibited a higher prevalence of *Wolbachia* than mosquitoes with oocysts (11.11%).

The *wAnM* and *wAnD* strains found naturally in *An. moucheti* and *An. demeilloni*, respectively, should have a lot of potential as candidates for use in *Wolbachia* biocontrol strategies in *Anopheles* to reduce the transmission of malaria (Figure 3). However, further studies are needed to investigate the ability to inhibit *Plasmodium* (36). Subsequently, the release of *Wolbachia*-infected males for population suppression should be performed if these strains are not ubiquitous over their native host populations (36). Conversely, if these strains are shown to inhibit *Plasmodium* transmission in their native hosts, as exhibited by Shaw et al. (56), selective release in areas showing lower prevalence in natural populations could lead to population replacement (36).

3.3 Effect of *Wolbachia* on *Plasmodium* in transfected *Anopheles* sp.

The possible transfection of *Wolbachia* for malaria control may be exploited when *Anopheles* species do not harbor natural *Wolbachia* infections. However, recent studies have reported the occurrence of *Wolbachia* in many species of *Anopheles*, which could compromise the current strategies.

Transinfection by embryonic microinjection in *Anopheles* populations is possible, even though *An. stephensi*, *An. arabiensis*, *An. gambiae*, and *An. funestus* harbor *Wolbachia* strains.

Both the *wMelPop* and *wAlbB* strains that transiently and somatically infected *An. gambiae* have been shown to significantly reduce the levels of *Plasmodium berghei* or *P. falciparum* oocyst infection in the mosquito midgut (64, 66). For instance, these

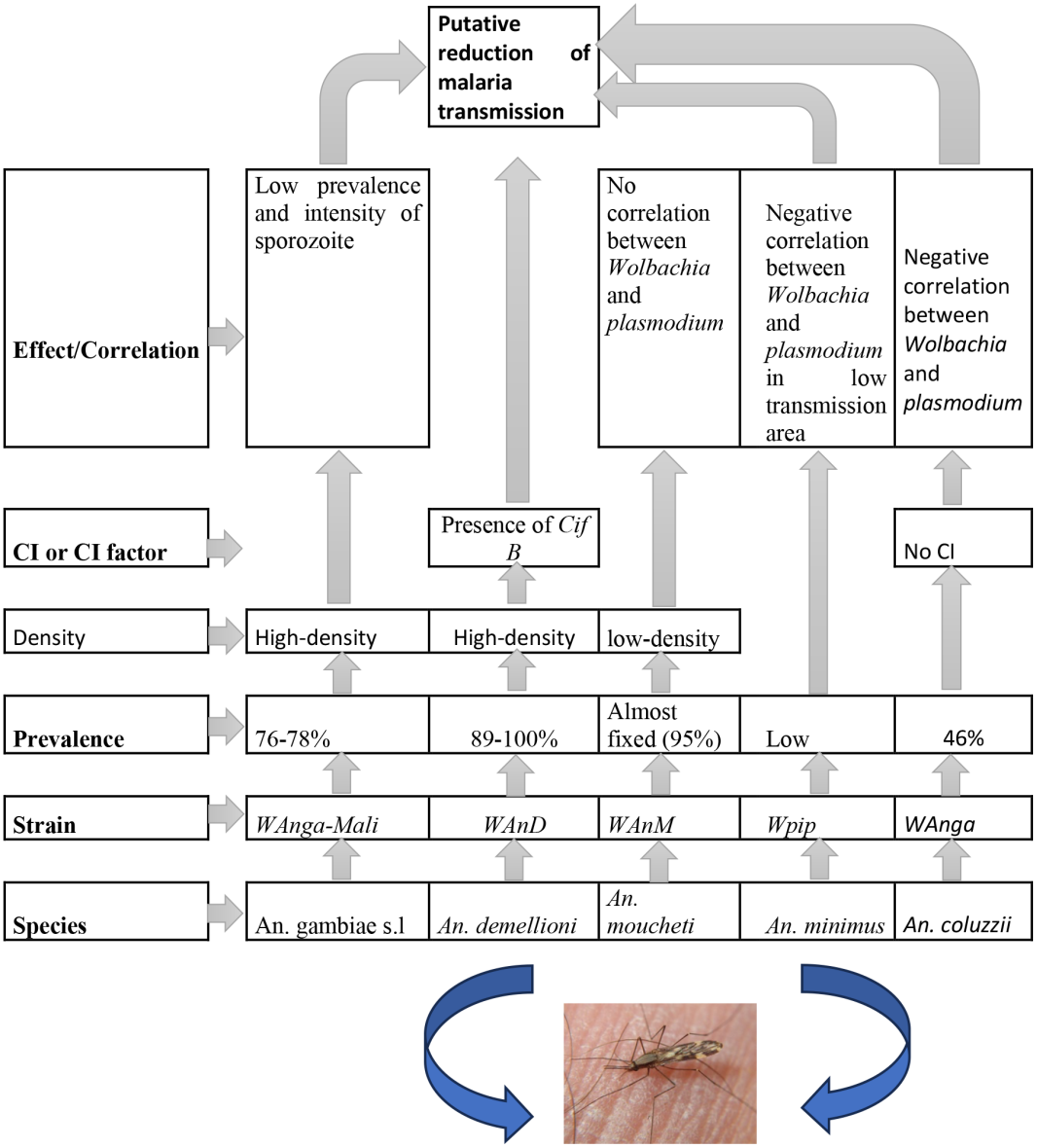

FIGURE 3
A synthetic view of role of *Wolbachia* on *Plasmodium* in natural Anopheles populations and guidelines for development of *Wolbachia*-based vector control.

strains could prevent the development of both *P. falciparum* oocysts and sporozoites. Bian and Joshi (63) showed that *An. stephensi* mosquitoes could be stably infected with the *Wolbachia wAlbB* strain microinjected through eggs (63) and could exhibit both the capability to induce high levels of CI and an impeccable maternal transmission. In addition, Gomes et al. (41), through infection design, found that *wAnga*-Mali infection impedes the maturation of sporozoites, thus reducing malaria transmission and opening new avenues for strategies to reduce disease transmission. The authors observed *Wolbachia* invasion in laboratory mosquito populations following several interbreedings of naturally uninfected males with infected females of *An. stephensi* populations. Moreover, *wAlbB* conferred resistance to *P. falciparum* in the mosquito.

However, *Wolbachia* transfection has not been implemented in field trials as *wAlbB* provides only partial blockage of parasite transmission (67). Other *Wolbachia* strains (e.g., *wMelPop* or *wAlbB*) that have shown significant results in *Ae. aegypti* infections and transinfections in *An. gambiae* could also impede *Plasmodium* transmission. Before their implementation, *Wolbachia* strains that could provide better blockage of malaria through *Anopheles* species need to be identified (67). According to Nazni et al. (68), if *Wolbachia* shows the ability to block malaria following transfers in *Anopheles* hosts, IC will also allow *Wolbachia* to spread into populations after releasing both males and females. Sustainable malaria control using *Wolbachia* strains such as *wAnD* and *wAnM* will finally require the transfection of strains capable not only of inhibiting *Plasmodium* parasites but also inducing CI without significant fitness costs (Figure 3). Experimental evidence has also shown the possible horizontal transfer of *Wolbachia* in *An. gambiae* (48) and *An. stephensi* (63, 69, 70).

4 *Wolbachia* and *Ae. aegypti* vector populations: prevalence and effect on pathogens

4.1 Prevalence of *Wolbachia* in *Ae. aegypti* populations

Although *Ae. albopictus* is known to be infected by *Wolbachia* at high frequencies, most findings have not mentioned their presence in *Ae. aegypti* populations for a long time.

The absence of natural infection is beneficial for both population suppression and replacement programs because any CI induced by *Wolbachia* infection should be unidirectionally incompatible with natural populations (27).

Most recently, the occurrence of *Wolbachia* in wild populations of *Ae. aegypti* has been reported in several locations including Florida (71), Malaysia (72), Thailand (73), Texas and the Philippines (74), India (75), New Mexico (26), and Panama (76) (Table 2).

To the best of our knowledge, low prevalence rates of *Wolbachia* have been reported in *Ae. aegypti* populations from Panama (0.2%) (76), Florida (4.35%) (71), and the Philippines (13.24%) (74), but reached 25% in Kuala Lumpur, Malaysia (72) (Table 2). Interestingly, the highest prevalence (57.43%) was reported in *Ae. aegypti* populations from New Mexico (26) (Table 2). Recently, in Meghalaya (Tura, India), this prevalence has reached 73.33% (78).

All of the studies reported variable levels of infection in populations, with a relationship between infections and several *Wolbachia* supergroups sometimes identified. Overall, all of the sequences found in these studies were closely related to those of *wAlbB* infection occurring naturally in *Ae. albopictus* (26, 71, 74, 75). It is possible that *Ae. aegypti* acquire *wAlbB* through environmental contamination as these species coexist with *Ae. albopictus* and share the same ecological niche. These species share several characteristics that provide them with adaptive advantages over others, making them successful invaders (79, 80). Both vectorial species exhibit high ecological plasticity under heterogeneous anthropic, climatic, and environmental conditions.

Carvajal et al. (74) detected a strain of *Wolbachia* (AAML: *Ae. aegypti* metropolitan Manila) from supergroups that were not ever found in *Diptera* species. Four samples belong to supergroups C and D and 85 samples are close to supergroup B. In addition to the detection of the presence of *Wolbachia* using PCR, other data are needed to confirm the presence of this bacterium in laboratory colonies, such as the loss of infection through antibiotic and assay on maternal transmission of *Wolbachia* (26, 75). The density of *Wolbachia* was quite low in the *Ae. aegypti* population included in the study by Kulkarni et al. (26), although a high frequency was estimated.

4.2 Transinfection of *Wolbachia* strains into *Aedes* populations for vector control

The *wAlbB* strain found in natural populations of *Ae. albopictus* has been successfully introduced into *Ae. aegypti* to provide an inherited infection line (81). Interestingly, the Toll and IMD pathways favor the establishment and maintenance of the *wAlbB* infection in this cell line. The *Wolbachia wMelPop* (32) and *wMel* (12) strains from *Drosophila* are suitable for infecting *Ae. aegypti* mosquito cell lines and have been extensively used in the transinfection of *Ae. aegypti* mosquitoes through embryonic microinjection.

Thus, eight *Wolbachia* strains (*wMel*, *wMelPopCLA*, *wMelCS*, *wRi*, *wAu*, *wAlbA*, *wAlbB*, and *wPip*) were successfully transfected with *Ae. aegypti* (81–84). All of them induced unidirectional CI when introduced into natural uninfected *Ae. aegypti*, except for the *wAu* strain (85). According to Ant et al. (84), the *Wolbachia* strain *wAu* provided a highly efficient virus transmission blockage in *Ae. aegypti*. *wMel*, *wMelPopCLA*, *wMelCS*, *wRi*, and *wAu* occur naturally in *Drosophila* species, whereas *wAlbA* and *wAlbB* are found in *Ae. albopictus* and *wPip* in *Culex* sp. is used to infect *Ae. aegypti*.

wAlbB and *wMelPopCLA* are often transinfected in *Anopheles* populations. In addition, successful transfers of *wAlbB* (inducing CI) from *Ae. albopictus* into *An. stephensi* have shown that *Anopheles* spp. can sustain *Wolbachia* infection (63).

Transinfections were also performed in *Ae. albopictus* (naturally infected with both *wAlbA* and *wAlbB*), in view of creating new crossing types. Bidirectional incompatibility was observed between the transfected and the naturally infected lines when both the *wPip* and *wMel* strains were introduced into *Wolbachia*-cured lines (86, 87). Moreover, a triple-infected (*wAlbA*, *wAlbB*, and *wPip*) *Ae. albopictus* line was created to express unidirectional CI when crossed with double-infected natural mosquitoes.

4.3 *Wolbachia*-mediated pathogen interference in *Aedes* mosquito populations

Most studies have shown that *Wolbachia* can inhibit pathogens caused by dengue, chikungunya, yellow fever, Zika virus, *Plasmodium* parasites, and filarial nematodes in infected *Ae. aegypti* or *Anopheles* sp. (22, 32, 63, 88) (Table 3). *Wolbachia* can reduce i) the virus transmission rate by decreasing the number of individuals with infection in the saliva; ii) the virus dissemination rate by decreasing the number of individuals with infection in the head or leg; and iii) the viral load by reducing viral gene copies (28, 63, 87, 89, 90). Moreover, it can reduce the parasite infection rate or parasite loads by i) decreasing the number of individuals infected with *the Plasmodium* parasite or ii) reducing the number of oocysts in the midgut from infected mosquitoes. In addition, *Wolbachia* can reduce parasite transmission by reducing the sporozoite load in the mosquito salivary gland (63, 64).

Ae. aegypti infected with *wMel* or *wAlbB* is less susceptible to disseminate infection of four serotypes of DENV via the salivary glands (93, 94).

Edenborough et al. (95) recently performed a comprehensive review focusing on three subcellular modifications: i) altered lipid homeostasis; ii) disruption of the intracellular membranes; and iii) changes to the host cell cytoskeleton that can boost *Wolbachia* to induce its antiviral effect.

TABLE 2 Prevalence of *Wolbachia* in natural *Aedes aegypti* vector populations.

Wolbachia strain	Supergroup	Infection type	Detection technique	Individuals tested (n)	Prevalence (%)	Site	Collection year	Authors
NA	B, C, D, J	Adults	16S rRNA sequencing	89	13.24	Manila, Philippines	2014–15	Carvajal et al. (74)
NA	C	Adults	16S rRNA sequencing	Unknown	Unknown	Thailand	2008	Thongsripong et al. (73)
NA	Unknown	Larvae	16S rRNA sequencing and electron microscopy	16	25	Malaysia, Kuala Lumpur	2013–14	Teo et al. (72) Balaji et al. (75)
wAegB	B	Natural	16S rRNA sequencing and electron microscopy	Unknown	Unknown	India	2019	Balaji et al. (75)
NA	B	Natural	16S rRNA sequencing	Unknown	NA	Florida	2014	Coon et al. (71)
wAlbB	B	Adults	16S rRNA sequencing	46	4.35	Florida	2016	Kulkarni et al. (26)
wAlbB	B	Adults	16S rRNA sequencing	148	57.43	New Mexico	2016	Kulkarni et al. (26)
Unknown	Unknown	Adults	16S rRNA high-throughput sequencing	Unknown	Unknown	Texas, USA	2015–2017	Bennett et al. (76)Hegde et al. (77))
wAlbB	B	Adults	16S rRNA sequencing	490	0.2	Panama	Unknown	Bennett et al. (76)

NA, not applicable.

Regarding these data, the *wAlB, wMel*, and *wmelPop* strains of *Wolbachia* have been selected for vector control strategies.

5 *Wolbachia*-based control strategies to reduce vector-borne disease transmission

There are two main *Wolbachia*-based strategies that result in the reduction of disease transmission: IIT or population suppression and population replacement.

The release of males artificially infected with the endosymbiont *Wolbachia* strains inducing CI and of females capable of transmitting infection led to population replacement and population suppression (Figure 1). Successful population replacement of *Ae. aegypti* transinfected by *Wolbachia* has been achieved in several countries (85).

After the release of infected mosquitoes in Australia, Malaysia, and the USA, *Wolbachia* infections have maintained a stable high frequency coinciding with a decrease in local dengue transmission (85, 96, 97). The success of both suppression and replacement programs is achieved by establishing community outreach programs with communications experts and educators (98).

5.1 Incompatible insect technique or population suppression

The IIT, which is based on bidirectional/unidirectional CI if a population is uninfected (35), consists in releasing *Wolbachia*-infected males, inducing CI and preventing the formation of viable offspring (i.e., in CI, *Wolbachia* induces the death of embryonic offspring from a cross between uninfected females and infected males) (18). This approach allows the sterilization of a large number of females able to transmit pathogens and reduces the total number of insect vectors (29). According to Zheng et al. (99) and Crawford et al. (100), the goal of IIT is to suppress target mosquito populations following mass releases of *Wolbachia*-infected male mosquitoes able to mate with wild-type females. This technique is analogous to the SIT, which is known to be efficient in controlling vector-borne diseases. Globally, CI then leads to a decline in population size targeted by fewer mosquitoes able to spread arboviruses.

TABLE 3 Viral inhibition induced by *Wolbachia* in *Aedes aegypti* vector populations.

Pathogen interference	*Wolbachia* strain	Mosquito species	Infection type	Pathogen (species)	CI phenotype	Infection effect (other phenotypes)	Reference
Viral inhibition							
	*w*AlbB	*Ae. polynesiensis*	Stable transfection	DENV	CI	Viral load reduction; declined virus transmission	Bian et al. (89)
	*w*AlbB	*Ae. aegypti*	Stable transfection	DENV	Unknown	Infection rate reduction, viral load reduction, and dissemination and transmission decline	Bian et al. (89)
	*w*Mel	*Ae. aegypti*	Stable transfection	CHIKV	Unknown	Viral load reduction, dissemination, and transmission decline	van den Hurk et al. (90)
	*w*Mel	*Ae. albopictus*	Stable transfection	CHIKV	Unknown	Reduction in virus transmission	Blagrove et al. (87)
	*w*AlB	*Ae. aegypti*	Stable transfection	DENV	Unknown	Inhibition of virus intracellular replication	Alkuriji et al. (91)
	*w*AlB	*Ae. aegypti*	Stable transfection	DENV	Unknown	Decreases the adult mosquito life span	Alkuriji et al. (91)
	*w*MelPop	*Ae. aegypti*	Stable transfection	CHIKV	Unknown	Infection rate reduction, viral load reduction, and dissemination decline	Moreira et al. (32)
	*w*MelPop	*Ae. aegypti*	Stable transfection	DENV	Unknown	Infection rate reduction, viral load reduction, and dissemination decline	Moreira et al. (32)
	*w*MelPop	*Ae. aegypti*	Stable transfection	WNV	Unknown	Infection rate reduction, viral load reduction, and dissemination and transmission decline	Hussain et al. (92)
	*w*MelPop	*Ae. aegypti*	Stable transfection	YFV	Unknown	Infection rate reduction, viral load reduction, and transmission decline	van den Hurk et al. (90)van den Hurk et al. (90)
	*w*AlB	*An. stephensi*	Stable transfection	*P. falciparum*	CI	Reducing parasite load and transmission	Bian and Joshi (63)
	*w*AlbB	*An. stephensi*	Stable transfection	*P. falciparum*	CI	Favored resistance in mosquitoes to *Plasmodium falciparum*	Bian and Joshi (63)
	*w*AlB	*An. gambiae*	Stable transfection	*P. berghei*	CI	Increase parasite	Hughes et al. (64)

CI, cytoplasmic incompatibility.

Suppression interventions require the large-scale deployment of millions of adult male mosquitoes across the country. In this technique, infected female mosquitoes are not released as they could accidentally spread *Wolbachia* into the targeted population. This also leads to less effective suppression as CI would not occur between the released males and females (101). Using populations infected by *Wolbachia* through introgression (102) and novel *Wolbachia* transinfections generated via microinjection (99, 103), population suppression can be achieved only through the release of *Wolbachia*-infected males, resulting in CI with wild females.

For population suppression interventions, the two major dengue vectors generally selected are *Ae. aegypti* (transfected with the *wAlbB* strain) and *Ae. albopictus* (naturally bi-infected with the native *wAlbA* and *wAlbB* strains and transfected with the *wPip* strain). In fact, large-scale deployment was performed in Fresno, CA, using *Ae. aegypti* (*wAlbB*), which led to a frequency above 95% of suppression of the target population, as well as a 78% decrease in the female numbers in Miami (100, 104). However, the authors did not investigate the incidence of dengue diseases, which would have allowed appreciating the success of these techniques. The same trend was observed in Guangzhou with triple-infected *Ae. albopictus* (*w*AlbA*w*AlbB*w*Mel with both native *wAlbA* and *wAlbB* strains and novel *w*Mel) (99). Recently, a transinfected *Ae. aegypti* population (*wAlbB-Tw*) in Taiwan has been shown to lead

to population suppression rates reaching up to 100% in laboratory experiments and 70% in semi-field experiments (105).

Interestingly, in Singapore, large-scale deployments combining IIT/SIT with *Ae. aegypti* (*wAlbB*) have allowed reaching above 93% of suppression of the target population, with a clear impact providing a proportion varying from 71% to 88% reduction in dengue cases (106). SIT combined with IIT could certainly improve the vector control results in reducing dengue cases.

Compared with other methods that use chemicals, which also reduce insect populations, the approach known as "self-delivering" has the potential to affect certain proportions of the vector population. In fact, releasing must be continued as it is possible that a low proportion of individuals not reached by chemicals can cause a speedy population recovery after termination of insecticide applications (107). However, repeated introductions of infected males are needed to prevent the recovery of mosquito populations.

Stakeholders appreciate the fact that biting females are not released and the self-limiting nature of suppression releases (108). This strategy has a limited effect on the release area beyond crashing the target population because male mosquitoes exhibit a short longevity and cannot spread *Wolbachia* (108). For these authors, the impact of this approach may be temporary if the target population promptly rebounds after release, requiring repeated interventions (108). In addition, due to inaccurate sex sorting, infected fertile females could be accidentally released into the targeted areas, which could result in replacement and the failure to suppress mosquito populations (101).

Suppression interventions need the development of space-optimized rearing facilities that can produce millions of adult mosquitoes each week (109). Mitigating the establishment of *Wolbachia* requires the combination of IIT with other strategies such as SIT.

5.2 Replacement

The population replacement strategy involves the release of both *Wolbachia*-infected male and female mosquitoes that may exhibit increased resistance to the pathogen and suppress the local uninfected population through CI (29). This strategy works as a rapid self-spreading method of *Wolbachia* into natural populations through the release of a small number of infected mosquitoes (12). This approach was implemented for dengue prevention in two locations in Australia. The release of between 10,000 and 22,000 individuals of *Wolbachia*-infected *Aedes* mosquitoes per week for 10 weeks in 2011 has shown interesting results in terms of the dengue elimination program.

In this strategy, individual males suppress the target population through CI and females spread *Wolbachia*. Population replacement interventions have a proven efficacy, with the rapid spread and long-term stability of *Wolbachia* infection in target populations at intermediate high frequencies (27). The successful establishment of *Wolbachia* has generally corresponded to a significant decline in dengue transmission in endemic areas (68, 110). Once *Wolbachia* has reached fixation in a population, replacement interventions could provide self-sustaining protection after a single deployment period.

Interventions were conducted in Latin America, Asia, and the Pacific through the World Mosquito Program or the *Wolbachia* Malaysia program. These programs provided mitigation results in terms of reductions in dengue incidence. Thereby, the release of *Aedes* species infected with *wMel* led to a post-release frequency of 73% in Yogyakarta (Indonesia) (111), of 100% over 2 years in Cairns, Queensland, Australia (97), a frequency above 80% in Townsville (96), and a frequency above 60% in Rio de Janeiro (110, 112). Consequently, these factors have reduced the incidence of dengue at certain proportions. In Kuala Lumpur, Malaysia, releases of the *wAlbB* strain of *Wolbachia* reached a post-release frequency of 98% during the 12 months of the survey, leading to a 40.3% reduction in dengue incidence (68). The introgression of *wMel* into *Ae. aegypti* populations reduced the incidence of symptomatic dengue and resulted in fewer hospitalizations due to dengue among participants from Yogyakarta, Indonesia (113). In the same area, following extensive community engagement and releases of *wMel*-carrying mosquitoes every 2 weeks for 13–15 rounds for 7 months in 2016–2017, 34 dengue cases from the release area and 53 from the control area (incidence of 26 *vs.* 79 per 100,000 person-years) were estimated (111). This corresponded in the regression model to a 73% reduction in dengue incidence coupled with the *Wolbachia* intervention (111).

One concern is that technology requires the release of biting females to ensure spread, which could be viewed negatively by stakeholders, even if these mosquitoes are not capable of transmitting arboviruses. The need for *Wolbachia*-infected mosquitoes to spread and persist in the environment remains the main challenge for replacement interventions. However, high fitness costs were often observed with the loss of *wMelPop* infection from *Ae. aegypti* populations after population replacement releases in Vietnam (114).

6 Implications of natural infections on *Wolbachia*-based disease control strategies

Both the population replacement and suppression techniques rely on novel *Wolbachia* infection types that induce CI in wild-type mosquito populations.

For a successful spread, the frequency of *Wolbachia* infection in the population must be above a threshold level, and CI can spread *Wolbachia* across the population, even though infection causes non-significant costs. If the initial prevalence of adult populations (0.43) is higher than the threshold infection rate (0.4), *Wolbachia* infection is expected to reach fixation over the next generations (115). The frequency of infection will likely decline until *Wolbachia* is suppressed from the population when the threshold is not reached (116).

The success of the population replacement or the suppression strategy may be constrained by the presence of natural *Wolbachia*

infections; therefore, potential crossing patterns between mosquitoes with novel *Wolbachia* infections and wild-type mosquitoes must be considered.

The high prevalence of *wAlbB* in natural *Ae. aegypti* populations in New Mexico and in infected colony obtained from wild-collected mosquitoes provided an opportunity to examine the role of *Wolbachia* in natural *Ae. aegypti* populations and to assess their interference with virus transmission (26).

With most natural infections found in wild-type populations of *Ae. aegypti*, the release of *Wolbachia*-infected male mosquitoes into an uninfected population will lead to CI. Reduced egg hatching from crosses between infected males and uninfected females favors infected females (27). The same authors also showed that putative crossing patterns between mosquitoes with novel *Wolbachia* infections inducing CI and mosquito populations with or without natural *Wolbachia* infections can lead to the possibility of four main outcomes. Subsequently, after the release of transinfected individuals, the following can occur: i) unidirectional CI via crosses between male mosquitoes with a novel *Wolbachia* infection and uninfected female mosquitoes; ii) no CI between novel and natural *Wolbachia* infections (compatible)—in this situation, population suppression is not possible; iii) bidirectional incompatibility that occurs between either males with a novel *Wolbachia* infection and females with natural *Wolbachia* infections or males with natural *Wolbachia* infections and females with a novel *Wolbachia* infection, which favor population suppression, as observed in *Ae. albopictus* (86); and iv) unidirectional CI may happen in favor of natural *Wolbachia* infection (when males from naturally infected mosquitoes mate with females with a novel *Wolbachia* infection) or in favor of novel infection (when males with a novel *Wolbachia* infection mate with females from naturally infected mosquitoes).

7 Concluding remarks

The occurrence of *Wolbachia* in natural populations at low or high frequencies raises the question about rethinking the *Wolbachia*-based control strategies. This led us to ask the following question: Does the natural occurrence of *Wolbachia* in *Anopheles* sp. and *Ae. aegypti* populations compromise the success of vector control strategies? In this paper, we aimed to answer this question and to propose guidelines for the development of *Wolbachia*-based vector control (Figure 3).

Several strains (*wAnga*-BF, *wAnga*-Mali, *wPip*, *wAnM*, and *wAnD)* of this endosymbiont in *Anopheles* populations have been reported. Among them, the *wAnM* and *wAnD* strains found in wild *An. moucheti and An. demeilloni* exhibit a lot of potential, suggesting their use in *Wolbachia* biocontrol strategies. In particular, the presence of *Wolbachia cifB* inducing CI provides promise in terms of vector control strategies and could contribute to reducing malaria transmission.

The occurrence of *Wolbachia* in *Anopheles* vectors does not exclude their infection by other strains known to produce CI. The transinfections of *Wolbachia* strains (*wMelPop* or *wAlbB*) in *An. gambiae* could provide significant outcomes in terms of reducing *Plasmodium* transmission. Figure 4 shows a synthetic view of the strains of *Wolbachia* transfected in mosquito populations and their impact on disease control. Laboratory transinfection of *Wolbachia* to *Anopheles* vectors of malaria was restricted to somatic tissues, and transinfection failures have generally been observed. However, previous research suggests that *Anopheles* disease vectors can support *Wolbachia* infections, which opens new opportunities for their use in disease suppression (67). Interestingly, *An. stephensi* mosquitoes stably infected with the *wAlbB* strain exhibited perfect vertical transmission, complete CI expression, strong pathogen blocking, and low fitness cost (63).

Moreover, the use of *Wolbachia* for malaria control will require creating stably infected lines of major malaria vectors, highlighting the protective effect of this bacterium against human malaria parasites such as *P. falciparum and Plasmodium vivax* (12, 41) (Figure 4). Finally, the release of *Anopheles* might be effective if further studies confirm that these *Wolbachia* strains are able to induce CI and express protective effects against *Plasmodium* species.

For the control of *Aedes* populations, the situation is quite different. Interestingly, following a large-scale deployment of the *wAlbB* strain, successful population replacement of *Ae. aegypti* infected with a novel *Wolbachia* strain has been achieved in Australia, USA, and Malaysia (68, 96, 97), coinciding with a decline in local dengue transmission.

To date, the occurrence of naturally infected *Ae. aegypti* requires thorough surveys for the detection of infection populations, including the choice of *Wolbachia* strain prior to the release of novel infections. Although most studies have reported the presence of *Wolbachia* in wild populations using 16S rRNA sequencing, additional studies, as described above (see *Section 3.1*), are needed to confirm the detection of this bacterium.

In addition to genome sequencing, the effects of natural infections (with higher prevalence) on some life history traits and vector competence must be examined as *Wolbachia* has useful properties that could aid in reducing virus transmission and/or decreasing population size (27).

According to Ross et al. (117), the population replacement and the suppression of *Wolbachia* are influenced by several factors: i) ecological effects (e.g., species composition and density in the breeding site) and the environment (i.e., temperature and competitors); ii) the *Wolbachia* variant (the ability to cause CI, fitness costs, and pesticide resistance); iii) disease pressure (virus incidence, serotype, and population immunity); iv) *Wolbachia* spread (mosquito density, the invaded area size, movement rate, and landscape structure); and v) operational issues (e.g., public engagement, quality assurance, the release technology, monitoring, and sexing, among others). These factors must be considered before the release of male-infected *Wolbachia* into targeted areas.

In any case, the unlikely presence of *Wolbachia* does not prevent the ongoing releases of this bacterium in various locations around the world, including Africa, which are aimed at reducing the transmission of vector diseases.

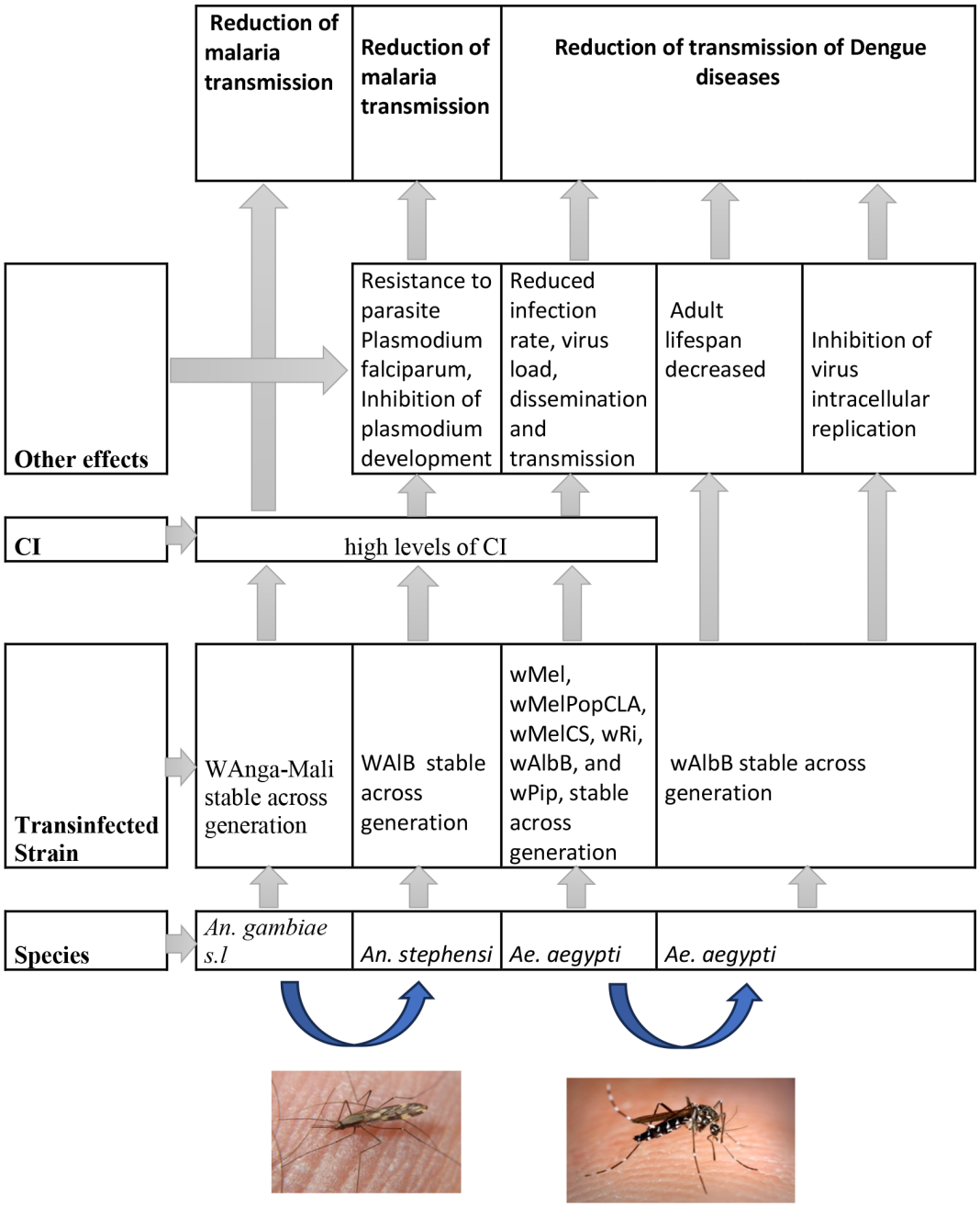

FIGURE 4
A synthetic view of strains of *Wolbachia* transinfected in mosquitoes populations and their effects on diseases control.

8 Future prospects

The prevalence and diversity of *Wolbachia* vary according to mosquito species. The major arboviruses vector, *Ae. aegypti*., is suspected to be infected by *Wolbachia*, while in the major malaria vector, *Anopheles* spp., most studies have reported their occurrence.

There is a real need for the ongoing releases to maintain control over mosquito populations. Further exploration of long-term strategies, such as genetic stability and ecological impacts, might be needed to improve the sustainability of these interventions.

In summary, future studies will consider further detailed mapping of *Wolbachia* strains in these two species in areas where dengue and malaria are endemic. Identifying factors such as the environmental conditions, local mosquito movement patterns (including immigration from neighboring areas with high mosquito density such as construction sites), and the nature of breeding sites need to be investigated.

Priority should be given to mosquito vectors that are the most difficult to control using the currently available methods. For this

purpose, *Wolbachia* could be used to target outdoor-biting and outdoor-resting species that can evade insecticide-treated nets and residual insecticide sprays.

Author contributions

OG: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. RKD: Methodology, Project administration, Resources, Validation, Visualization, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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