

Full Length Research Paper

Endosymbiont bacterium *Wolbachia*: Emerged as a weapon in the war against mosquito-borne diseases

Agersew Alemu

School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar, P. O. Box 196, Gondar, Ethiopia.

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Because of climate change and failure of the existing methods of control of vector borne diseases and vector are increasing. Mosquito species are the main vectors of human pathogens causing malaria, dengue, filariasis, chikungunya, yellow fever and West Nile. There are no well-organized methods and tools of controls of vector and vector borne diseases, since no efficient vaccines or drugs are available. Despite years of intense effort to control them, many of these diseases are increasing in prevalence, geographical distribution and severity, and options to control them are limited. Currently, efforts focused on the control of vector populations. During recent years, the endosymbiont bacterium has been well-documented and has led to suggestions that these could be used to control pests and therefore diseases. *Wolbachia* is perhaps the most renowned insect symbiont, primarily due to its ability to manipulate insect reproduction and to interfere with major human pathogens therefore providing new avenues for pest control. *Wolbachia* are common intracellular bacteria that are found in arthropods and nematodes. These alphaproteobacteria endosymbionts are transmitted vertically through host eggs and alter host biology in diverse ways, including the induction of reproductive manipulations, such as feminization, parthenogenesis, male killing and sperm-egg incompatibility. *Wolbachia* strains can invade and sustain themselves in mosquito populations, reduce adult lifespan, affect mosquito reproduction and interfere with pathogen replication. *Wolbachia* can also provide direct fitness benefits to their hosts by affecting nutrition and development, influencing fecundity or oogenesis and providing resistance to pathogens. For instance, infection of *Anopheles gambiae* with both *wMelPop* and *wAlbB* reduced the oocyst burden of *Plasmodium falciparum*, compared to uninfected control mosquitoes. In addition, similar study observed that the *wMelPop* strain inhibited development of *Plasmodium berghei*; however, the *wAlbB* strain was found to enhance development of *P. berghei*.

Key words: Malaria, *Aedes aegypti*, Chikungunya, dengue, drosophila, *Wolbachia pipientis*, vector-borne diseases.

INTRODUCTION

Vector-borne diseases occur in more than 100 countries, mainly within the tropics, with the annual, global death

*E-mail: yigeremagersew@gmail.com; agersewalemu@yahoo.com.

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rate in millions (McGraw and O'Neill, 2013). A variety of vector-borne diseases, which often coexist in the same environments, impose a heavy burden on human populations, in developing countries mainly in tropical and subtropical zones. Besides the direct human suffering they cause, vector-borne diseases are also a significant obstacle to socioeconomic development (KKeiser et al., 2005). Insect-borne diseases, mostly those transmitted by mosquitoes, are among the most important causes of mortality and morbidity in humans (World Health Organization, 2008). The occurrence of mosquito-borne diseases such as malaria, lymphatic filariasis, dengue fever, Chikungunya, West Nile virus, yellow fever and Japanese encephalitis are rise annually due to human travel, rapid urbanization and failures of preventative public-health measures (Adams and Kapan, 2009).

Recent study reported that Vector borne diseases (VBD) are increasing because of the climate change and failure of the existing methods of vector control and vector borne diseases. Moreover, a sudden increase of VBDs is reported due to many factors like insecticide resistant vector population, drug resistant parasite population and lack of effective vaccines against the VBDs, and thus insecticides are no longer a sustainable control method of vector and vector-borne diseases due to environmental pollution, public health hazard and insecticide resistant vector population (Gupta et al., 2012). Despite the existence of a variety of vector control measures, disease incidence is usually growing, and therefore there is an urgent need to develop new and effective control approaches (McGraw and O'Neill, 2013), because no effective vaccines or treatments against vector bore diseases exist (Wilder et al., 2010) and control methods are failing to prevent the global increase in the incidence of the disease (Ricci et al., 2011a). These new and effective strategies should be used in combination with existing control techniques, and in this context the bacterium *Wolbachia pipientis* has proven to be a promising option, given its ability to limit pathogen growth in numerous dissimilar mosquito pathogen combinations (Kambris et al., 2009; Bian et al., 2010; Glaser and Meola, 2010; Bian et al., 2013), and currently being applied in the field in Australia, Vietnam and Indonesia.

Wolbachia was believed to be members of an uncommon and unimportant bacteria genus until the early 1990s. However, after the introduction of molecular typing techniques *Wolbachia* were found to be prevalent and familiar in arthropods filarial nematodes. A recent meta-analysis estimated that more than 65% of bug species harbor *Wolbachia*, making it among the most plentiful intracellular bacteria genus so far revealed, infecting at least 10 insect species alone (Hilgenboecker et al. 2008).

Wolbachia are members of the order Rickettsiales, a dissimilar group of intracellular bacteria having parasitic, mutualistic and commensal relationships with their hosts. Although, the related genera (like *Anaplasma*, *Ehrlichia* and *Rickettsia*) infects (Werren et al. 1994), *Wolbachia*

do not routinely infect vertebrates. *Wolbachia* have attracted substantial interest in the past decade primarily because of their huge abundance, fascinating effects on hosts, which ranges from reproductive manipulation to mutualism, and potential applications in pest and vector born diseases control (Werren et al., 1994). *Wolbachia* is a vertically-transmitted bacterial endosymbiont of arthropods that is able to influence its host's reproductive system and thus spread quickly through wild populations (Werren et al., 2008). *Wolbachia* was originally identified in the ovaries of the mosquito *Culex pipiens* (Hertig and Wolbach, 1924), and recent studies have estimated that 40% of terrestrial arthropod species are infected with *wolbachia* (Zug and Hammerstein, 012). Based on Multi-Locus Sequence Typing (MLST) (Baldo et al., 2006), *Wolbachia* is recently divided into eight monophyletic "supergroup" lineages (A-H) (Lo et al., 2007), with new hosts being discovered constantly (Wang et al., 2010; Vasquez et al., 2011).

METHODOLOGY

Qualitative method was used to evaluate the significance and problems related to vector born diseases. Authors reviewed different journals, reports (WHO recommendations), and related documents. Accessible materials were browsed from internet sources which were published from 1924 to 2014. The following sites and search engines were used: HINARI, Medline (Pubmed), Google scholar, and Science Direct. The selection process is as illustrated in Figure 1.

WOLBACHIA INVASION OF MOSQUITO POPULATIONS AND HOST BIOLOGY

A study in India demonstrated that paratransgenic based approach can be used effectively, where dengue, chikungunya, malaria and filariasis are prevalent (Gupta et al., 2012). *Wolbachia* are common intracellular bacteria that are found in many terrestrial arthropods and nematodes. These alpha proteobacteria endosymbionts are transmitted vertically through host eggs and alter host biology in different ways, including the induction of reproductive manipulations, such as feminization, parthenogenesis, male killing and sperm-egg incompatibility. They can also move horizontally across species boundaries, resulting in a widespread and global distribution in diverse invertebrate hosts (Werren et al., 2008). *Wolbachia* are highly adapted for living within invertebrate cells, which probably partly explains their wide distribution (Serbus and Sullivan, 2007).

A similar study reported that the effects of *Wolbachia* infection for example feminization of genetic males; parthenogenetic induction, which results in the development of unfertilized eggs; the killing of male progeny from infected females; and cytoplasmic incompatibility (CI) also called sperm-egg incompatibility, and collectively, these strategies are referred to as reproductive parasitism.

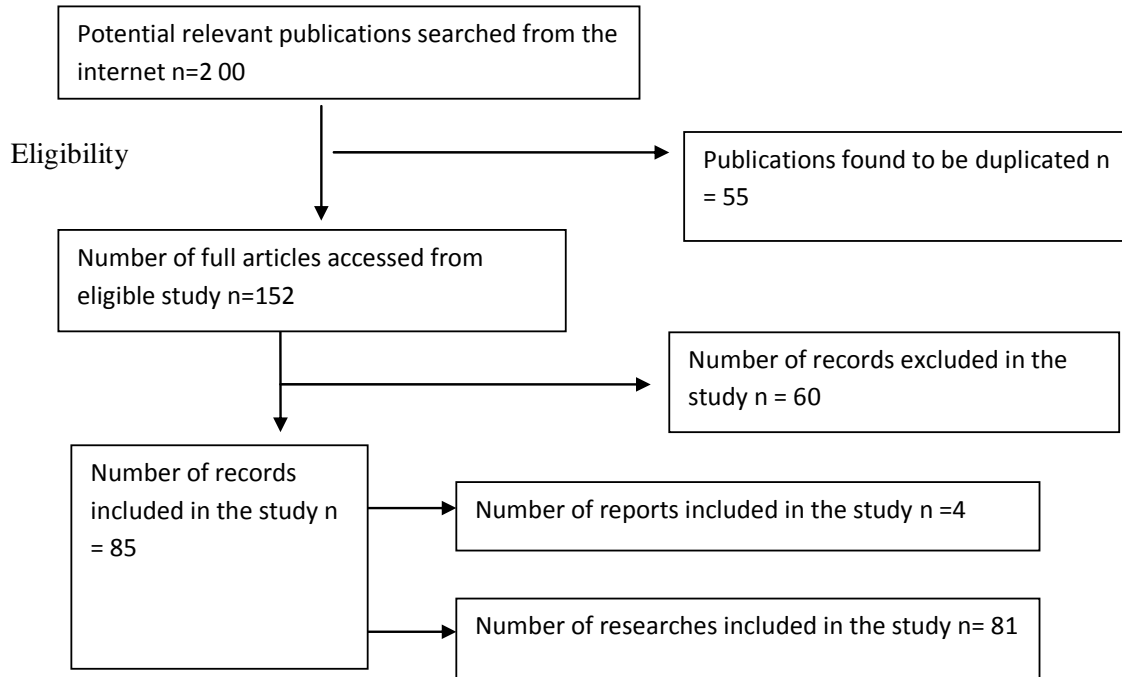


Figure 1. Flow diagram showing reviewed documents.

Furthermore, *Wolbachia* have also evolved mutualistic interactions with their filarial hosts, and show a range of other host effects (Serbus and Sullivan, 2007). Different up to date studies showed that the symbiotic bacterium *Wolbachia* is an attractive agent for vector-borne pathogen control. The ability of *Wolbachia* to manipulate host reproduction and spread into arthropod populations (Werren et al., 2008), together with the recently recognized ability to inhibit diverse pathogens (Hedges et al., 2008; Kambris et al., 2009; Moreira et al., 2009b; Kambris et al., 2010; Hughes et al., 2011), open an opportunity for its use in controlling vector and vector-borne disease. Numerous *Wolbachia* based control strategies are being investigated (Iturbe-Ormaetxe et al., 2011; McGraw and O'Neill, 2013; Bourtzis et al., 2014), with some studies having progressed to field trials (Walker et al., 2011; Hoffmann et al., 2011).

The endosymbiont bacterium *Wolbachia* influences host physiology positively (Brownlie et al., 2009; Kambris et al., 2010), which is recognized for parasitism that alters host reproductive success, including cytoplasmic incompatibility (CI) (Werren et al., 2008). CI is the most studied reproductive modification induced by *Wolbachia* and results in embryonic lethality when uninfected females are crossed with *Wolbachia* infected males. In a population composed of infected and uninfected individuals, only infected females can mate successfully with infected and uninfected males (Werren et al., 2008). When two *Wolbachia* strains exist in a population, bidirectional CI can result in incompatibility between individuals

carrying different strains, while individual females infected with multiple strains (superinfected) can mate with all males and produce infected progeny (Engelstadter et al., 2009). In both CI types, *Wolbachia* is expected to sweep through populations due to higher reproductive fitness because of the higher proportion of successful matting's between infected or super infected females relative to the uninfected ones. However, not all *Wolbachia* strains cause CI and strength of CI expression (penetrance) can be altered by *Wolbachia* density or transmission efficiency (maternal transmission fidelity) (Unckless et al., 2009).

Given the influence of *Wolbachia* on host fitness, the potential impact of *Wolbachia* on host population genetic variability and geographical patterns is substantial. Since *Wolbachia* is maternally transmitted, other maternally transmitted organelle (example, mitochondria) hitchhike with *Wolbachia* infections (Turelli et al., 1992; Rasgon et al., 2006). Even though simulations indicate that CI-based spread of *Wolbachia* sweeps are more likely to involve repeated initial infections via horizontal transmission (Egas et al., 2002; Jansen et al., 2008), most studies of CI associated *Wolbachia* sweeps find it associated with low mitochondrial DNA (mtDNA) variation and with many hosts infected (Nunes et al., 2008). Theoretical models suggest that host dispersal or migration, and genetic background (Duron et al., 2007; Mouton et al., 2007) can influence these sweeps (Keeling et al., 2003; Telschow et al., 2005; Flor et al., 2007; Engelstadter et al., 2009). Factors that control *Wolbachia* density, such as nutrient availability or temperature (Hurst

et al., 2000; Dutton and Sinkins, 2004), indirectly influence CI-based sweeps, because at high, *Wolbachia* densities maternal transmission fidelity and CI expression are stronger than those at low *Wolbachia* densities. Although the mechanism is not well known, *Wolbachia*-induced CI has received considerable attention as a mechanism to control insect vectors and diseases. *Wolbachia* is responsible for inducing a number of reproductive modifications that enables its spread and maintenance in natural populations (Saridaki and Bourtzis, 2009; Guruprasad et al., 2013).

Recently, there has been a considerable raise in *Wolbachia* research related to the interactions of *Wolbachia* with its hosts and its impact on parasite transmission. Fruit fly *Drosophila melanogaster* *Wolbachia* strains can invade and sustain themselves in mosquito populations, reduce adult lifespan, affect mosquito reproduction and interfere with pathogen replication. Such endosymbiotic bacterial strains have been introduced in *Aedes aegypti* (*Ae. aegypti*) mosquito populations to reduce their life span, thereby reducing the extrinsic incubation period. The other prospect of exploiting *Wolbachia* is using its ability to interfere with viruses and parasites. *Wolbachia* is known to interact with a wider range of pathogens in transfected mosquitoes including dengue and chikungunya viruses (Brelsfoard and Dobson, 2011).

A major advantage of *Wolbachia*-based control approach for mosquitoes is that CI acts as a self-spreading mechanism for *Wolbachia* to rapidly invade populations from the release of relatively small numbers of individuals. *Wolbachia* provides a biological method to manipulate mosquito populations and reduce disease transmission (Iturbe-Ormaetxe et al., 2011). Since *Wolbachia* based control methods are mostly environment-friendly than insecticide-based techniques, findings have encouraged researchers to aid in the control of mosquito-transmitted diseases. Similarly, identical *Ae. aegypti* lines infected and uninfected with wMelPopwolbacha strain were compared to determine whether differences in gene expression between the two lines were related with the life-shortening phenotype, wMelPop induces an up-regulation of the mosquito's innate immune system and that its presence inhibits the development of filarial nematodes in the mosquito. Once again, wMelPop could be used in control programs to eradicate lymphatic filariasis and other MBDs (Zielinski et al., 2008).

WOLBACHIA TRANSFER INTO AEDES AEGYPTI MOSQUITOES

Dengue fever is the most important arboviral disease in humans; 40% of the population of the world in more than 100 countries is at risk of infection and an estimated 50 to

100 million cases occur annually (Guzman and Kouri, 2002; WHO, 2009). Dengue (DENV) is primarily transmitted by the infectious bite of a female *A. aegypti* mosquito and to a much lesser extent, *Aedes albopictus* (Lambrechts et al., 2010).

Wolbachia infections are relatively common in mosquitoes (Kittayapong et al., 2000) including *C. pipiens* (Yeap et al., 2010), *quinquefasciatus*, *Aedes fluviatilis* (Moreira et al., 2009b) and *Aedes albopictus* (Moreira et al., 2009a). The main vectors for dengue fever (*A. aegypti*) and malaria (*Anopheles* spp.) are not naturally infected by *Wolbachia*. Approaches that use *Wolbachia* for the control of diseases transmitted by uninfected, naive insects rely on the successful establishment of stable *Wolbachia* infections, usually by embryonic microinjection of *Wolbachia*-infected cytoplasm or *Wolbachia* purified from infected insect hosts. To create stably transinfected lines, embryo injections must target the region near the pole cells in pre-blastoderm embryos to incorporate *Wolbachia* into the developing germline and favour the transmission of *Wolbachia* to offspring. Several *Wolbachia* strains have been transferred across phylogenetically distant insects and, importantly, the phenotypes induced by these strains in their native hosts are generally also expressed in the newly infected hosts (Iturb et al., 2011). *Wolbachia* transinfection experiments are more likely to be successful when the donor and recipient organisms are closely related. In line with this, the transfer of wMelPop from its natural host, *D. melanogaster*, into the dengue fever vector *A. aegypti* was achieved in our laboratory after *Wolbachia* was first maintained by continuous passage in *A. albopictus* *in vitro* cell culture for almost 4 years (McMeniman et al., 2009).

Wolbachia adapted to a mosquito intracellular environment, facilitating transinfection *in vivo*. After microinjection of thousands of *A. aegypti* embryos, two stable wMelPop-CLA (cell-line-adapted) lines with maternal transmission rates of approximately 100% were generated (McMeniman et al., 2009). wMelPop-CLA-infected mosquitoes showed an approximately 50% reduction in adult lifespan, compared with their uninfected counterparts (McMeniman et al., 2009). The halving of adult mosquito lifespan and the high *Wolbachia* maternal transmission rates were also maintained in more genetically diverse outbred mosquitoes, and larval nutrition did not affect the life-shortening ability of the wMelPop-CLA strain (Bian et al., 2010; Yeap et al., 2010). The wMelPop-CLA infection is widespread in *A. aegypti* tissues, with high bacterial densities in the head (brain and ommatidia), thorax (salivary glands, muscle) and abdomen (fat tissue, reproductive tissues and Malpighian tubules) (Moreira et al., 2009). Wide distribution across tissues has been found in other transinfected mosquitoes, such as *A. aegypti* infected with the wAlbB strain from *A. albopictus* (Bian et al., 2010). By using quantitative Polymerase Chain Reaction (PCR) and Western blot analyses, this strain was also found in

reproductive tissues, midgut, muscles and heads, in both native *A. albopictus* (Dobson et al., 1999) and the transinfected *A. aegypti* (Bian et al., 2010), although the densities are not as high as those found in *A. aegypti* infected with wMelPop-CLA.

WOLBACHIA INTERFERENCE WITH VIRUSES AND PARASITES

A key element in the use of *Wolbachia* for the control of insect-borne disease has been the discovery that some *Wolbachia* strains can interfere with insect viruses in *Drosophila* and human pathogens in mosquitoes. Interestingly, the presence of *Wolbachia* interferes with a wider range of pathogens in transinfected mosquitoes including nematodes and bacteria (Kambris et al., 2009), viruses such as DENV and Chikungunya (Moreira et al., 2009a; Bian et al., 2010), as well as the avian and rodent malaria parasites *Plasmodium gallinaceum* (Moreira et al., 2009b) and *P. berghei* (Kambris et al., 2010). Natural *Wolbachia* strains that infect mosquitoes have also been shown to induce resistance to viruses as in *Culex quinquefasciatus* mosquitoes, that are resistant to West Nile virus (Glaser and Meola, 2010) although, this resistance seems less pronounced in comparison to transinfected *Wolbachia* strains such as wMelPop-CLA (Moreira et al., 2009a). The mechanisms by which some *Wolbachia* strains interfere with a variety of pathogens remain unclear. One assumption is that pathogen interference is partly mediated by the induction of antimicrobial peptides and pre-activation of the innate immune response in the insect (Kambris et al., 2009; Moreira et al., 2009b; Kambris et al., 2010). The presence of wMelPop-CLA *Wolbachia* in *A. aegypti* induced the expression of several immune effect or molecules, including cecropin, defensin, thio-ester containing proteins and C-type lectins (Moreira et al., 2009b). When the wMelPop strain was transiently injected into adult *Anopheles gambiae*, several immune genes were upregulated, as shown by whole-genome arrays (Kambris et al., 2009), resulting in the inhibition of *Plasmodium* development (Kambris et al., 2009).

WOLBACHIA INTERFERES WITH PLASMODIUM PARASITES

Malaria is a disease caused by infection of *Plasmodium* protozoan parasites by the bite of anopheline mosquitoes which results in an estimated 1 to 2 million deaths per year, taking a dramatic toll on health and socioeconomic development in affected areas (World Health Organization, 2008). Another similar study also indicated that malaria-transmitting anopheles mosquitoes are the deadliest animals on this planet, causing the death of more than 600,000 people each year and endangering

the lives of half of the world's population (World Health Organization. World Malaria Report: World Health Organization 2013). As a result, malaria remains one of the most critical public health challenges for Africa despite intense national and international efforts (WHO, 2012).

As different researchers point out that the current insecticide-based control strategies to stop malaria transmission by targeting the mosquito vector which are limited by the rapid spread of insecticide resistance (Ranson et al., 2011). Moreover, insecticide-based control strategies target only indoor feeding and resting populations, with the use of insecticide treated bed nets and the application of indoor residual sprays, respectively. The use of *Wolbachia* endosymbionts has been proposed as an alternative to chemical strategies because of the ability of *Wolbachia* bacteria to rapidly invade insect populations through CI, (Walker and Moreira, 2011) and successful *Wolbachia* invasions in field settings have been demonstrated in the case of the dengue and yellow fever vector *Aedes aegypti* (Hoffmann et al., 2011).

Recent proof have shown that *Wolbachia* infections of anopheles vectors limit the development of the plasmodium parasites that causes malaria (Kambris et al., 2010; Hughes et al., 2011; Bian et al., 2013; Murdock et al., 2014) makes these bacteria a particularly attractive tool for the control of both endophagic and exophagicanophelines mosquito. Long-standing limitations concerning the introduction of *Wolbachia* into laboratory colonies of anopheles mosquitoes have been recently overcome (Bian et al., 2013); however, the usefulness of this system for the control of anopheles populations has been undermined by the apparent absence of natural infections. Indeed, *Wolbachia* strains have been detected in many insects (Hilgenboecker et al., 2008).

A recent study confirmed that *P. falciparum* development in *Anopheles gambiae* (*A. gambiae*) is suppressed transiently as a result of *Wolbachia* infection. This reproductive parasite is known to indirectly support and up-regulate the insect-host immune system and suppress the pathogen (Pinto et al., 2012). *Wolbachia* limits the spread of numerous human pathogens by manipulating their reproduction and immunity. In anopheles mosquitoes, experimental *Wolbachia* infections can reduce plasmodium numbers in the laboratory; however, natural *Wolbachia* infections in field anophelines have never been reported. A study in Burkina Faso, West Africa has shown evidence of *Wolbachia* infections in anopheles *gambiae*. Sequencing of the 16S rRNA gene identified *Wolbachia* sequences in both female and male germlines, and also determined that these sequences are vertically transmitted from mother to offspring. Whole-genome sequencing of positive samples suggests that the genetic material identified in *A. gambiae* belongs to a novel *Wolbachia* strain related to but distinct infecting

other arthropods. The evidence of *Wolbachia* infections in natural anopheles populations promotes further investigations on the possible use of natural *Wolbachia* anopheles associations to limit malaria transmission (Baldini et al., 2014).

Interestingly, *Wolbachia* can protect insects from pathogens and limit their ability to transmit mosquito-borne pathogens (Iturbe et al., 2011). This effect was first observed where naturally *Wolbachia* infected *Drosophila* were protected against fungal and viral pathogens (Panteleev et al., 2007). More complex pathogens are also susceptible to *Wolbachia* mediated pathogen interference. wMelPop infected *A. aegypti* has a reduced capacity to transmit *Brugiapahangi* (a rodent filarial model) (Kambris et al., 2009; van den Hurk et al., 2012) and *P. gallinaceum* (avian malaria). Importantly, it shows the inhibition phenotype transfers to *Plasmodium* species of human relevance. Transient somatic infection of *A. gambiae* with both wMelPop and wAlbB reduced the oocyst burden of *P. falciparum*, the major causative agent of human malaria, compared to uninfected control mosquitoes (Hughes et al., 2011).

A similar study observed that the wMelPop strain inhibited the development of *Plasmodium berghei* and the mouse malaria model; however, the wAlbB strain was found to enhance development of *P. berghei* (Hughes et al., 2012). Recently, symbiont-mediated refractoriness to *Plasmodium* was also observed in *A. stephensi* artificially-infected with a stable *Wolbachia* infection (Bian et al., 2013). In particular, it was shown that the wAlbB infection can significantly inhibit *P. falciparum* infection at both oocyst and sporozoite stages (Bian et al., 2013). Interestingly, the wPip strain was seen to protect *C. pipiens* mosquitoes against *Plasmodium relictum* induced mortality, increasing the lifespan of *Wolbachia* infected mosquitoes (Zélé et al., 2012). These data suggest that the pathogen protection phenotype is dependent on the specific *Wolbachia* parasite combination and serving as a warning that not all host *Wolbachia* combinations will retard parasite development.

Nematode associated *Wolbachia* show a general concordance between the phylogeny of the bacteria and the phylogeny of their hosts, and all these *Wolbachia* have evolved mutualisms with their hosts. This pattern is also found with many other vertically inherited endosymbionts, such as *Buchnera aphidicola*, the obligate intracellular symbionts of aphids (Funk et al., 2000). By contrast, *Wolbachia* that participate in symbiotic relationships with arthropods have a range of phenotypic effects on their hosts, and generally behave as reproductive parasites. There is no concordance between the phylogeny of arthropod *Wolbachia* and the phylogeny of their hosts, which is an indicative of extensive lateral movement of *Wolbachia* between host species. Furthermore, resolving the relationships between strains is further complicated by extensive recombination, even from strains among some super

groups (Baldo et al., 2006; Baldo and Werren, 2007; Hilgenboecker et al., 2008).

WOLBACHIA INHIBITS DENGUE AND CHIKUNGUNYA VIRUS REPLICATION IN MOSQUITOES

Evidence from several recent studies indicates that a strain of life-shortening *Wolbachia* has been detected in the fruit fly *Drosophila*. This virulent *Wolbachia* strain wMelPop is responsible for the shortening of life span in *D. melanogaster* (Min and Benzer, 1997). In *Drosophila*, the wMelPop and another closely related *Wolbachia* strains have the ability of protecting against RNA virus infection by delaying the mortality of flies infected with a range of pathogenic viruses (Hedges et al., 2008; Teixeira et al., 2008). The *Wolbachia* wMelPop infection in *D. melanogaster* induces antiviral response to the *Drosophila* C virus in their hosts, cricket paralysis, Nora and Flock House viruses (Osborne et al., 2009), West Nile virus (Glaser and Meola, 2010), as well as the fungus *Beauveria bassiana* (Panteleev et al., 2007). These observations in *Drosophila* have made researchers to introduce this bacterial strain into the dengue virus mosquito vector *A. aegypti* artificially. The introduction of wAlbB strain reduces the proliferation of dengue virus when compared with uninfected mosquito population. The *Wolbachia* strain not only reduced the virus replication but also reduced the adult life span. The life-shortening *Wolbachia* exerts its effect by altering the extrinsic incubation period of dengue virus, thereby inhibiting its transmission to new host. Meanwhile life-shortening *Wolbachia* may offer a new technology to control the chikungunya virus as well. These results may offer a potential new method to control vector-borne diseases like dengue and chikungunya virus from *A. aegypti* (Moreira et al., 2009).

Mosquitoes infected with wMel showed significantly reduced rates of chikungunya infection and dissemination to the salivary glands compared to controls, but only in the oral exposure experiments. Chikungunya also showed limited dissemination in wMelPop-CLA-infected mosquitoes following oral exposure (Moreira et al., 2009), suggesting that both strains of *Wolbachia* may be useful candidates for release in chikungunya control programs. By contrast, yellow fever (YFV) was much less likely to infect and disseminate in *A. aegypti* infected with wMelPop-CLA compared to wMel strains. The virus was also less likely to replicate in wMelPop-CLA infected mosquitoes, with very high virus loads detected in wMel-infected *A. aegypti*.

These experiments suggest that wMelPop-CLA infected mosquitoes may be the best candidates for YFV biocontrol programs, but were unable to determine the extent of virus replication following oral exposure rather than intrathoracic inoculation. Because virus inhibition with some *Wolbachia*-virus combinations does not

appear to be complete, it is essential that epidemiological models be utilized to establish the threshold virus inhibition necessary to minimize and prevent transmission in the field (van den Hurk, 2012). The chikungunya strain was isolated from a patient visiting Melbourne, Australia in 2006 and contained the alanine to valine mutation in the membrane fusion glycoprotein E1 gene (E1-A226V) that has been linked to increased infectivity in mosquitoes, especially *A. Albopictus* (Druce et al., 2007).

WOLBACHIA PIPIENTIS AND DISEASE CONTROL

The potential application of the symbiotic bacteria *Wolbachia pipientis* to the control of mosquito-borne diseases has emerged as a recent addition to the arsenal of weapons against mosquitoes. It is more environmentally friendly than insecticide-based approaches and more cost effective. In recent years, there is an interest in *Wolbachia bacterium* as a means by which to control insect-transmitted diseases. However, *Wolbachia* induced cytoplasmic CI was proposed as a tool for *Culex* mosquito control as early as 1967 (Laven, 1967) and there were trials to eradicate mosquitoes in India in the 1970s (Curtis and Adak, 1974), but although there has been some field testing, it has never been operationally implemented. *Wolbachia*, the most-common known endosymbiotic microbe in the biosphere, is thought to infect up to 76% of the estimated 2 to 5 million insect species on earth (Hilgenboecker et al., 2008). The success of these small (0.5 to 1µm) intracellular bacteria has been attributed to their ability to induce a series of reproductive distortions in their hosts to increase the reproductive success of infected females, thus enhancing the maternal transmission of *Wolbachia* (Werren et al., 2008). These traits include transforming genotypic males into phenotypic females, modifying male sperm so that females cannot produce progeny unless they mate with a male infected with the same strain of *Wolbachia*, or inducing the parthenogenetic reproduction of females (Stouthamer et al., 1999).

FITNESS OF WOLBACHIA INFECTED MOSQUITOES

Wolbachia can also provide direct fitness benefits to their hosts by affecting nutrition and development (Brownlie et al., 2009; Hosokawa et al., 2010), influencing fecundity (Aleksandrov et al., 2007) or oogenesis (Dedeine et al., 2001) and providing resistance to pathogens (Hedges et al., 2008; Moreira et al., 2009; Osborne et al., 2009; Bian et al., 2010; Glaser and Meola, 2010; Kambris et al., 2010). *Wolbachia* infected mosquitoes can only spread and invade uninfected mosquito populations if the fitness cost of infection is less than the fitness advantage that CI provides for the infection to spread. Pathogen protection

might also provide a fitness advantage to *Wolbachia* infected mosquitoes that will assist their spread in the field. Apart from the reduction in lifespan, some of the fitness effects induced by the wMelPop-CLA infection in *A. aegypti* include an increase of metabolic rate and activity in the mosquito (Evans et al., 2009), and a fecundity cost. The latter is detected as a steady reduction in hatch rates after the first gonothropic cycle, probably due to an impaired ability to feed as the mosquitoes age (Turley et al., 2009). Another significant effect of wMelPop-CLA infection in *A. aegypti* is the reduction of egg survival during periods of embryonic quiescence (McMeniman and O'Neill, 2010). This might be a desired control mechanism for population suppression in areas with pronounced wet/dry seasonality, by preventing the next generation of mosquitoes from hatching after the dry season.

The ability of mosquitoes infected with wMelPop-CLA to feed on human hosts has been tested by looking at the volume of blood they have ingested, their ability to probe successfully, and other aspects of their biting behavior (Moreira et al., 2009). *Wolbachia* does not affect the response time of mosquitoes to humans, but its presence reduces the number and size of blood meals taken. wMelPop-CLA *Wolbachia* also induced behavioural changes in old mosquitoes termed 'shaky' or 'bendy', in which the proboscis bends and is unable to pierce the skin; 65% of 35 day old insects showed the bendy phenotype (Turley et al., 2009). *Wolbachia* infected *A. aegypti* produce smaller volumes of saliva, which contain the same levels of the anti-platelet-aggregation enzyme and apyrase, as uninfected mosquitoes (Moreira et al., 2009).

Despite the ability of the wMelPop-CLA strain to induce strong CI and interfere with dengue virus (DENV) replication in transinfected *A. Aegypti* mosquitoes, the fitness effects produced in its host might be counterproductive to, or even completely block, the establishment of this strain in natural populations of mosquitoes (Turelli, 2010). Alternative, less-virulent strains might therefore be required. In *Drosophila*, viral interference is induced by several *Wolbachia* strains that are closely related to wMelPop (Hedges et al., 2008; Osborne et al., 2009) suggesting non-life-shortening strains with more desirable invasion characteristics which would also affect transmission of dengue fever.

Although not related with this review, the wickerhamomyces anomalus yeast, which has been indicated as a symbiont of some mosquito vector species, has been found in the midgut and reproductive organs of the host (Ricci et al., 2011b). This mosquito symbiont can be cultured in cell free media and thus may be a good candidate for the expression of effect or molecules in the midgut of mosquito vectors. A recent study describes the use of the transgenic *Metarhizium anisopliae* fungus to inhibit malaria transmission, abolishing parasite development within the mosquito (Ricci et al., 2011). Interestingly,

another study investigates a bacterium of the genus *Chromobacterium* (Csp_P), which was isolated from the midgut of field-caught *Aedes aegypti*. It is reported that Csp_P can effectively colonize the mosquito midgut when introduced through an artificial nectar meal, and it also inhibits the growth of other members of the midgut microbiota. In addition, Csp_P colonization of the midgut tissue activates mosquito immune responses, and Csp_P exposure dramatically reduces the survival of both the larval and adult stages. Importantly, ingestion of Csp_P by the mosquito significantly reduces its susceptibility to *P. falciparum* and dengue virus infection, thus compromising the mosquito's vector competence. The anti-pathogen and entomopathogenic properties of Csp_P render it a potential candidate for the development of malaria and dengue control strategies (Ramirez et al., 2014).

CONCLUSION

The ability of some *Wolbachia* strains to reduce the lifespan of *A. aegypti*, invade mosquito populations through the induction of CI in particular, interfere with the replication of a variety of pathogens, which has placed this bacterium at the frontline of new approaches targeting mosquito-borne diseases in an environmentally friendly manner. During the last two decades, surprising progress has been achieved in the field of *Wolbachia* symbiosis. The prevalence and diversity of the symbiont has been studied in all major classes of insects including mosquito genera that are known to contain major disease vector species such as *Aedes*, *Anopheles* and *Culex* genera. *Aedes* (but not *A. aegypti*) and *Culex* mosquito species were found to be naturally-infected. The *Wolbachia* induced extended phenotypes, most notably cytoplasmic incompatibility and pathogen interference, and other symbiont effects on naturally-infected and transinfected species have been intensively studied, resulting in the transfer of *Wolbachia* research from the laboratory to the field.

Anopheles species, naturally uninfected, have been found reluctant to support *Wolbachia* transinfections until very recently. Recent study reported that *A. stephensi* can support the wAlbB *Wolbachia* strain, can express CI and block pathogen transmission (Bian et al., 2013). This is a major breakthrough which opens the way for the application of *Wolbachia* based approaches for the control of *Anopheles* mosquitoes and malaria. However, it should be noted that in many malaria endemic areas, multiple malaria vectors and genotypes thereof are present, and *Wolbachia* based technologies may work only in areas with a single vector species (Walker and Moreira, 2011). In addition, it should be noted that pathogen interference works for newly transinfected species only; attenuation and replacement may pose a significant problem for this technology. Different recent studies have shown that the most important goal for the

Wolbachia based biocontrol approach to mosquito-borne-disease control is to transfer *Wolbachia* into anopheline mosquitoes, the most-common vectors of human malaria.

Conflict of interest

The authors declare that they have no conflicts of interest.

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