Chapter

# The Potential for Wolbachia-Based Mosquito Biocontrol Strategies in Africa

*Femi Ayoade and Tosin S. Ogunbiyi*

## Abstract

The three foremost medically important mosquito species of public health importance belong to the genera Anopheles, Aedes, and Culex. The Anopheles mosquito is the most important in the transmission of human malaria, while members of the genera Culex and Aedes are more important in the transmission of arboviruses. Reducing the number of competent vectors has been identified as a logical method for the control of malarial and arboviral vector-borne diseases. This chapter provides an update on the potentials of biological vector control, specifically the release of endosymbionts to help limit the reproductive capability of mosquitoes, thereby reducing the population of the disease vectors in Africa. There are examples of successful suppression of mosquito-borne diseases by the establishment of Wolbachia in mosquito populations elsewhere, however, there has been no such report from the African continent. Although the establishment of stable maternally transmissible Wolbachia in natural mosquito populations is yet to be achieved in Africa, this area of research is experiencing unprecedented progress within the past decade. Many of the research efforts are hereby highlighted, including the problems and prospects of establishing a Wolbachia-based biocontrol program in Africa.

**Keywords:** Wolbachia, cytoplasmic incompatibility, integrated vector control, paratransgenesis

## 1. Introduction

Of the three foremost medically important mosquito genera of public health significance, namely, *Anopheles*, *Aedes,* and *Culex*, the *Anopheles* mosquito is most important in the transmission of human malaria while members of the genera *Culex* and *Aedes* are more important in the transmission of arboviruses [1]. Since it is impractical to eliminate mosquitoes, reducing the number of competent vectors is a logical target for controlling malaria and arboviral vector-borne diseases. For some mosquito-borne arboviruses such as West Nile, chikungunya, dengue, Zika, and so on that lack licensed vaccines or viable therapeutics, in addition to the problems posed by the ever-plastic plasmodium parasite that continues to exhibit resistance to even the most potent combined therapeutic agents, this may actually be the only option left [2].

The present chapter is focused on the potential of using a proven biological vector control method, specifically the release of mosquitoes infected with endosymbionts that help to limit the reproductive capability of mosquitoes to reduce the population of the disease vectors in Africa. Many insect species are infected by intracellular bacteria, and these are known to sometimes exert deleterious effects on the host insects. *Wolbachia* is perhaps the best-known example of intracellular bacteria that can drastically reduce the reproductive capability of several insect species, particularly disease-bearing mosquitoes. *Wolbachia* is an alpha proteobacterium first described in *Culex pipens* by *Wolbachia* and for this reason, was named *Wolbachia pipientis* [3]. Similarly, *Wolbachia* has been isolated from *Drosophila*, *Aedes albopictus*, and other insect species; in fact, reports have shown that these bacteria only infect invertebrate hosts and are naturally found in more than 50% of all arthropod species and in several nematodes [4].

Today, *Wolbachia* is still relevant in biological control programs due to its potential as a safe vector for spreading cytoplasmic incompatibility and other means of reproductive isolation among disease-bearing vectors, such as induction of parthenogenesis, feminization, and male-killing [5, 6]. In recent times, there are notable examples of successful establishment of *Wolbachia* in mosquito populations aimed at suppressing mosquito-borne diseases [7–10]. Remarkably, the Australian *Wolbachia* project tagged "eliminate dengue" (www.eliminatedengue.com) has shown that *Wolbachia* bacteria can prevent Dengue virus (DENV) transmission in mosquitoes without high fitness costs. Moreover, a virulent *Wolbachia* strain in Drosophila melanogaster fruit flies (named *w*MelPop) is known to lower the lifespan of its host significantly. It has been shown to shorten the lifespan of mosquitoes [11].

In addition, a closely related avirulent *w*Mel strain was found to protect their native hosts, Drosophila fruit flies, against infection by pathogenic RNA viruses [12, 13]. Recent reports indicate that such strains that provide similar or better characteristics deployable in preventing the capacity of viruses to replicate in the vector or the ability to incapacitate the vector (such as *w*MelPop and *w*Mel strains) exist in Africa. An example is a report by the insect vector research group at the African Centre of Excellence for the Genomics of Infectious Diseases (ACEGID) laboratory recently reported finding *Wolbachia* in Ede (Osun State), which is the first report from Nigeria [14].

*Wolbachia* has been reported from countries in West Africa and even from *Anopheles* species initially thought not to be naturally infected by *Wolbachia*. African countries from which natural mosquito infections by *Wolbachia* have been reported include Burkina Faso [15]; Ghana, the Democratic Republic of the Congo (DRC) [5, 16], and Mali [17]. Since success rates of *Wolbachia* infections have been attributed to the relatedness of the donor and recipient hosts [16], the present chapter focuses on the great potential in developing indigenous strains of *Wolbachia* that might be used in artificial infections that can reduce the capacity of wild mosquito populations to reproduce and transmit human pathogens in Nigeria and possibly elsewhere in Africa. Moreover, the artificial infection of mosquitoes may produce inhibitory effects on arboviruses and *Plasmodium* parasites as observed in Australia and elsewhere in Asia [18, 19].

## 2. The microbiome of mosquitoes

As a result of their interactions with biotic and abiotic factors in their ecosystem, mosquitoes internalize diverse consortia of microbes, which have been shown to have a significant effect on this insect's physiology. Microbes belonging to diverse life

forms (bacteria, protists, viruses, and yeasts) have been identified and characterized as established or occasional members of the mosquito microbiome. Some members of this symbiotic microbiota can either be beneficial (e.g. dietary supplementation, enhancement of digestive mechanisms, tolerance of environmental perturbations, protection from parasites and pathogens, and maintenance and/or enhancement of host immune system homeostasis) or detrimental (reducing the fitness or life span of

| Diseases | Mosquitoes | Global Burden |
|---|---|---|
| Dengue | *Aedes aegypti, Aedes albopictus* | • More than 2.5 billion people (over 40% of the world's population) are at risk.<br>• More than 100 million dengue infections are reported yearly.<br>• An estimated 500,000 people with severe dengue require hospitalization each year.<br>• About 2.5% of those affected died. |
| Yellow fever | *A. aegypti* and *Haemagogus* | • About 200,000 cases of illness and 30,000 deaths are reported yearly.<br>• Number of reported cases has been on the increase for the past two decades due to declining population immunity and deforestation. |
| Chikungunya | *A. aegypti* and *A. albopictus* | • In 2005–2006, an outbreak in Reunion Island (a French territory in the Indian Ocean) affected about one-third of the population (266,000 of 775,000 inhabitants).<br>• The 2006 outbreak spread to other countries in South-East Asia resulting in 1.4 million reported cases.<br>• In December 2013, the first cases of local transmission of Chikungunya were detected in the WHO Region of the Americas, the Caribbean island of Saint Martin. |
| Zika virus | *A. aegypti* | No information on global disease burden (as at 28th of April, 2018). |
| Japanese encephalitis (Found in Asia) | *Culex tritaeniorhynchus* | Causes an estimated 50,000 cases and 10,000 death yearly, mostly in children less than five. |
| West Nile Virus | *A. albopictus, Culex* | No information on global disease burden (as at 28th of April, 2018). |
| Malaria | *Anopheles* (more than 60 known species can transmit diseases) | • Malaria transmission occurs in 91 countries.<br>• In 2016, an estimated 216 million cases were reported with an estimated 445,000 deaths.<br>• About 3.4 billion people are at risk. |
| Lymphatic Filariasis (LF) | *Anopheles* (more than 60 known species can transmit diseases) | • More than 120 million people are currently infected.<br>• 40 million of those infected are disfigured and incapacitated by the disease.<br>• LF afflicts more than 25 million men with the genital disease and more than 15 million people with lymphoedema. |

*Source: WHO [19]; Available from: www.who.int/news-room/fact-sheets/detail/malaria [Accessed on: 12 December, 2021].*

**Table 1.**
*Diseases transmitted by various mosquito species and their global disease burden.*

their host); while other members of this community are of medical significance to the host on which the insect feeds on [20–28].

The microbes that constitute the microflora of the mosquito are the causal organisms of infectious diseases of global public health importance. Consequently, the process of diseases vectoring by a mosquito may not be viewed as a deliberate act but rather an accidental act that happens during a normal blood meal, necessary for reproduction. Interestingly, the selective feeding pattern seen in mosquitoes creates a possibility of having infectious agents from an "unusual host" introduced into a completely susceptible new host. This is the basis for most emerging infectious diseases that are of zoonotic origin; mosquito, once infected, remains infectious for life [29]. According to the World Health Organization, the infectious diseases of public health importance that are vectored by mosquitoes include dengue, yellow fever, chikungunya, zika virus, japanese encephalitis, west nile virus, malaria, and lymphatic filariasis [19]. A list of these diseases, the global disease burden, and their mosquito vectors are presented in **Table 1**.

## 3. Vector control as a means of disease control

In the early twentieth century, vector control emerged as one of the main methods of disease control. During this era, environmental management of breeding sites, including larviciding, was employed in the reduction of mosquito vectors. Around the 1950s, insecticides (most especially DDT) were introduced and used extensively. Interestingly, by the 1970s most mosquitoes had developed resistance to these insecticides, and on discovering the environmental hazard these chemical agents place on the ecosystem, its continuous use was frowned upon [30]. This new development led to the re-evaluation of vector control programs. In 1982, WHO recommended an integrated vector control (IVC) program based on the Axtell principle of integrated pest management [30]. The Axtell principle is founded upon the combination of biological control methods such as the introduction of exotic natural enemies, larvivorous fish, microbial agents with source reduction methods such as intermittent irrigation, water level management, landfilling, channeling, and draining in combination with the use of chemicals, including insect growth regulators, adulticide, and larvicides integrated with the use of personal protection methods, such as bed nets and repellents, concurrently with health education in the various communities at the schools, on television and mass media. Of all the mosquito control components highlighted in the IVC strategy, only biological control has not been implemented successfully in Africa, although some baseline data necessary for implementation are recently being generated. Most of the problems preventing the incorporation of biological control methods in IVC strategies in Africa are due to limited capacity, as the implementation of biocontrol methods requires a high level of technical capability. Moreover, since other control measures like chemical control have inherent limitations of environmental toxicity and the emergence of resistant strains of the vector, IVC programs in Africa have not been so successful, largely due to the lack of mastery of the biological control component.

## 4. Biocontrol in IVC programs

Biological control methods employ the use of natural enemies like fish, insects, protozoa, fungi, bacteria, and viruses to reduce the population of mosquitoes or

reduce their vectorial competence. The two most widely employed mosquito biological vector control methods include larvicides and larvivorous fish. The use of small-sized fishes that feed on mosquito larvae has the advantages of being cost-effective, environmentally safe, and long-term effective control measures against different varieties of mosquito species. On the other hand, this has some limitations such as it requires a large number, takes about 2 months (not suitable for quick intervention), less effective in waters with floating garbage or vegetation. Sometimes birds and in some African communities, humans prey on the fishes as some of the larvivorous species are delicacies in these African communities. Examples of larvivorous fish include *Gambusia spp* and *Poecilia spp* (Guppy) [31]. On the other hand, the use of bio-larvicides involves the use of bacteria for the control of mosquito larvae. *Bacillus sphaericus* and *Bacillus thuringiensis* H 14 are the two most widely used bio-larvicide usually available as granules and wettable powder, which contain lyophilized bacteria, spores, and toxic crystals. The mechanism of biolarvicide control employed by *B. thuringiensis* H 14 and *B. sphaericus* involves the production of endotoxins (Cry4A, Cry4B, and Cry11A) which result in gut paralysis and leakage of gut contents into the body cavity, which finally results in death due to osmotic shock. Toxins of *B. sphaericus* have been shown to be more effective in polluted water (polluted water is characteristic of *Culex* breeding sites). They are environmentally safe and do not pose any threat to humans and their livestock but are expensive [31–33].

The third mosquito biological vector control method is paratransgenesis involving the use of native bacteria flora in disease vectors to express effector molecules capable of interfering with pathogen transmission. Paratransgenesis begins by the screening of internal microbiota of the vector to isolate symbiotic bacteria that are genetically modified to express effector molecules, after which they are again reintroduced into the vector that is now introduced into the wild where they produce the desired effect [34–36]. Understanding bacteria diversity in mosquitoes is the bull's eye in paratransgenic control of mosquitoes, and this requires a detailed knowledge base of the biology of the local mosquitoes and their microflora. To be effective, the bacterial population in the local mosquito populations are screened in order to identify bacteria that are consistent and persistent in all generations and across a variety of mosquito species. For this reason, a bacterium is considered suitable as a paratransgenesis agent when it has an effector molecule that produces the desired effect; an exocytotic mechanism to discharge the effector molecule on its cell surface; and ability to survive long enough to produce the expected amount of effector molecules in the mosquito [37–39].

Gaio et al. [40] investigated the contribution of midgut bacteria to blood digestion and egg production in *Ae. aegypti*. Findings from this study showed that eradication of gut bacteria resulted in a slower growth rate and decline in fecundity. The researchers concluded that alteration of gut flora should be further investigated as a new approach for preventing the transmission of pathogens and controlling mosquito populations.

Paratransgenic management of infectious disease and their insect vector is considered to have advantages of increased bacteria number after ingestion of blood (by the vector), which will invariably cause an increase in the secretion of effector molecules by the genetically modified bacteria. The expected outcomes of paratransgenesis include a reduction in mosquito's vectorial competence; obstruction of pathogen transmission; loss of fecundity in mosquito (non-viable eggs and alteration of embryogenesis); and eventual death of the mosquito [41–45].

## 5. *Wolbachia*: a paratransgenic agent

*Wolbachia* is an obligate intracellular gram-negative bacterium belonging to the family Rickettsiales; it is known to be part of the microbiota of insects, isopods, nematodes, and mites (**Figures 1** and **2**). As an obligate parasite, they infect the cytoplasmic vacuoles of their host cell, including gonads. *Wolbachia* can be vertically transmitted or maternally inherited and are therefore considered as potential targets for paratransgenic systems [48, 49]. Many mosquito species (especially those

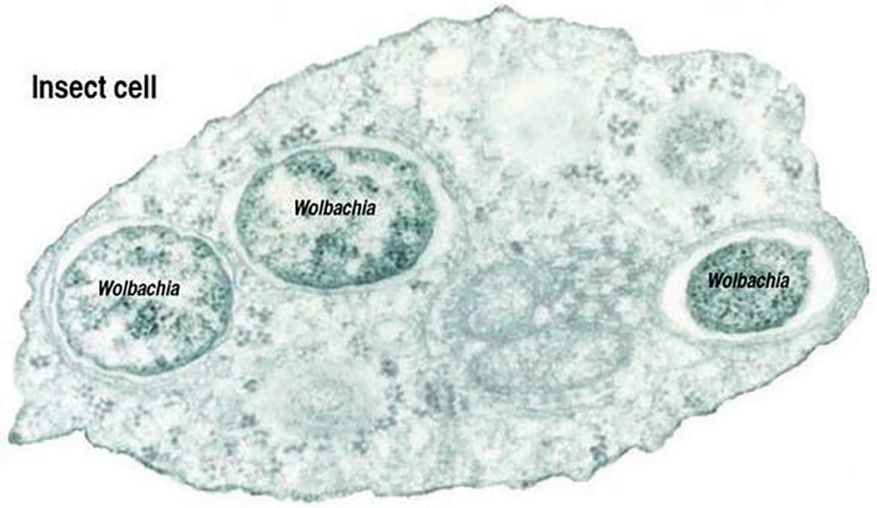


**Figure 1.**
*Electron micrograph of* Wolbachia *within an insect cell [46].*

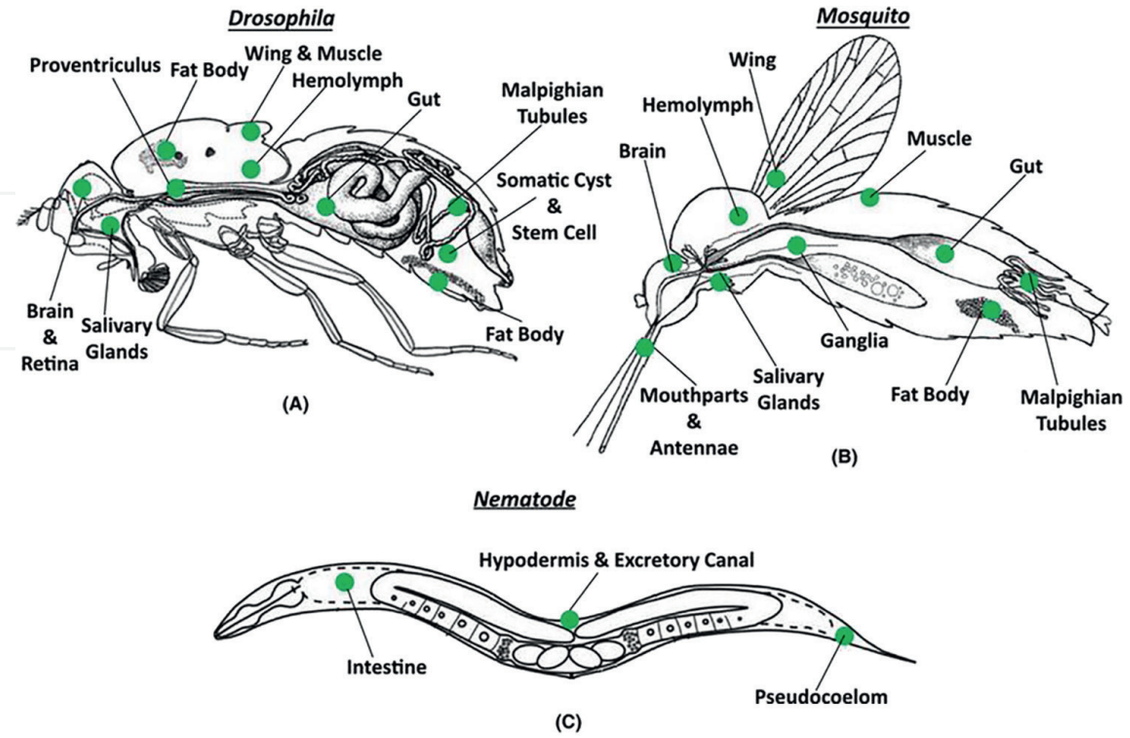


**Figure 2.**
*Distribution of* Wolbachia *(in green) in somatic tissues of various hosts as detected by PCR and fluorescent cytology [47].*

of epidemiological importance) are known to be susceptible to *Wolbachia* infection; however, the prevalence of this bacterium is notably high in wild *Ae*. *albopictus* and *Cx*. *pipiens* population. Different phylogenetic strains of *Wolbachia* induce distinct extended phenotypes in the mosquito they infect; the effect induced by this bacterium in their host can be cytoplasmic compatibility, incompatibility or compatibility in only one direction [50]. The persistence of *Wolbachia* population through the generation of mosquitoes is known due to the bacterium's ability to induce a severe selective pressure that rapidly drives its transovarial transmission [51, 52].

Basic approaches to using *Wolbachia* for paratransgenic control of vectors of infectious diseases include:

1. Direct insertion of the transgene into the bacterium's genome and the use of cytoplasmic incompatibility to suppress the targeted vector population.

2. Fixing the transgene on cytoplasmic elements of the host that are co-inherited with the bacterium; and

3. Transformation of the host genome coupled with the use of the bacterium's cytoplasmic incompatibility mechanism to insert this gene into other members of the target population [48].

The ability of *Wolbachia* to induce transovarian transmission of itself is considered a major boost in paratransgenic systems. This means once the bacterium has been introduced into the host, they can persist for several generations in the insect; hence, once introduced, there is no need for subsequent re-introduction [53, 54]. Interestingly, the effect induced by *Wolbachia* is species-dependent [55]. For example, infected *Aedes aegypti* with different strains of *Wolbachia* resulted in three outcomes: shortened lifespan [54]; reduced susceptibility to dengue or chikungunya virus and *Plasmodium* infection [18]; and, depending on the infecting strain, cytoplasmic incompatibility was observed, with apparent high horizontal transmission and no high fitness cost [54]. The foregoing underscores the importance of capacity development in the areas of research and laboratory-based surveillance systems in ensuring the successful introduction, establishment, and maintenance of *Wolbachia* populations wherever paratransgenesis is used as a biocontrol method as part of an integrated vector control strategy.

## 6. Wolbachia in Africa

The presence of *Wolbachia* in wild *Anopheles gambiae* mosquitoes was first demonstrated by Baldini et al. [15] in Burkina Faso. Hughes et al. [56] demonstrated that a stable maternally transmissible *Wolbachia* population can be achieved in *An. gambiae* and *An. stephensi* by suppressing other members of the insect microbiota with the use of antibiotics. Furthermore, Shaw et al. [5] demonstrated the ability of the *wAnga* strain to stably infect reproductive tissues (ovaries), and certainly somatic tissues where the *Plasmodium* development occurs, with the potential to effectively compete for resources or upregulate the immune response to kill the malaria parasite. Similar results were reported in Mali with a new anopheline *Wolbachia* strain (*wAnga*-Mali) [17]. Moreover, reports have shown that there are native *Wolbachia* infections in 16 out of 25 wild African *Anopheles* species, including both vectors and non-vectors of malaria

[16, 57]. These reports and more recent reports [58] confirm that natural *Wolbachia* infection in anopheline mosquitoes is more common than expected and underscores the need for further studies in the diversity of anopheline *Wolbachia* strains towards identifying suitable strains that may serve to impede the development of *Plasmodium* parasites in mosquitoes and other *Wolbachia* strains associated with non-malaria vectors that are responsible for other infectious agents of health importance.

## 7. Conclusions and recommendations

The fact that more researchers in Africa in recent years are looking and finding *Wolbachia* [14, 15, 17, 58] in African mosquito populations is a welcomed change, unlike previously when there was no activity in this area of research in Africa. However, none of these strains are yet to be found to confer Cytoplasmic Incompatibility (CI), a condition needed to spread rapidly in natural populations and as such disrupt disease transmission. In laboratory experiments, environmental factors such as temperature and availability of food have been shown to affect the expression of CI. For example, rearing males at temperatures higher than 25°C and low levels of nutrition was found to lead to increases in cytoplasmic incompatibility [59], although the environmental factors were found to be mediated by bacterial density. On the other hand, it may be expedient to consider developing a genetically modified *Wolbachia* to induce CI or to select *Wolbachia* strains that can spread efficiently in natural mosquito populations.

Five strategic areas of development have been identified as critical to the establishment of impactful IVM programs in Africa; enhanced advocacy, intra, and inter-collaboration, integrated approach, capacity building, particularly human resource development [60]. Apart from these strategic areas, basing decisions increasingly on local evidence, and community involvement and empowerment to ensure sustainability have also been identified as key components of successful IVM programs in Africa [61]. There are wide variations to the extent of adoption and promotion of these prerequisites to successful IVM among the African countries with the consequent variations in success rates. While some countries are still grappling with the consolidation of strategic and operational frameworks, others have advanced to the point of adopting IVM as a national policy, and have implemented its key elements in different measures of success [61].

Using IVM strategies, progress has been achieved with increased intervention coverage, reduced risk of transmission, and reduced VBD burden, particularly for malaria, in some African countries, including, Namibia [62], Swaziland [63], Botswana [64], Zambia and Zimbabwe [65]. These successes however may not be entirely attributed to vector control alone but also to effective case management, community mobilization, and sensitization, including changing climatic and environmental factors. These kinds of successes can be replicated in Africa if the best practices are adopted by more countries in Africa.

Developing the required technical capacity and infrastructure for entomological surveillance is another area of focus that needs to be developed in Africa, particularly, sub-Saharan Africa. This has been identified as a major challenge for most African countries [62]. Although it may take some time to develop this capacity, reports show that in countries where targeted training of entomological technicians have been conducted, such as Burundi, Eritrea, Guinea, and Zambia, the corresponding reduction in the malaria burden by up to 99% was achieved in some cases [60].

Moreover, since vector control of mosquito-borne diseases, must rely on insecticides as its backbone, particularly via long-lasting insecticidal nets (LLIN) and indoor residual spraying (IRS), the development of insecticide resistance has been identified as a potentially limiting factor in IVM programs [66]. On the other hand, combination innovative approaches including genetically modified or transinfected mosquitoes (Wolbachia-based), durable wall linings, mosquito traps such as eave tubes and entomopathogenic bacteria traps, odor-baited traps, attractive toxic sugar baits, spatial repellents, and entomopathogenic fungus-impregnated targets are expected to be effective when used in support of the application of insecticides "backbone" [62].

In conclusion, a great potential for IVM has been demonstrated in various regions of Africa, particularly in the area of malaria vector control [67, 68]. However, deploying IVM strategies for effective vector control in Africa will require sustained funding, removal of governmental bureaucracy, strategic planning and human resource development, and synergy among stakeholders, including community-based groups and their collaboration with nongovernmental organizations, international and national research institutes, and various government ministries.

## Author details

Femi Ayoade[1,2*] and Tosin S. Ogunbiyi[3]

1 Department of Biological Sciences, College of Natural Sciences, Redeemer's University, Ede, Osun State, Nigeria

2 African Center of Excellence for the Genomics of Infectious Diseases (ACEGID), Redeemer's University, Ede, Osun State, Nigeria

3 Department of Biological Sciences, Mountain Top University, Prayer City, Ogun State, Nigeria

*Address all correspondence to: ayoadef@run.edu.ng

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