RESEARCH PAPER

The effects of antibiotics on the reproductive physiology targeting ovaries in the Asian tiger mosquito, Aedes albopictus

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Abstract
Mosquitoes have adapted to various environmental conditions. Symbionts with mosquitoes impact this adaptation in different environments. In the field, mosquitoes could get exposed to antibiotics during their developmental period, which could reduce or eliminate their symbiotic microbes. However, the side effects of the antibiotics on the ovary and reproductive physiology of the Asian tiger mosquito, Aedes albopictus remains unknown. In this study, we investigated the effects of tetracycline and combinations of rifampicin and tetracycline at environmentally acceptable levels on the reproductive physiology of ovaries in Ae. albopictus. Rifampicin and tetracycline in combination reduced the hatching rate and fertility of Ae. albopictus compared to the untreated control group. These antibiotics induced histopathological damage and reactive oxygen species production in the ovaries. The combination of antibiotics decreased the expression of surface protein of Wolbachia (WSP) in Ae. albopictus. Additionally, the expression of Toll like receptor 2 (TLR2) and Myd88 were triggered by the combinations. The findings demonstrate the detrimental effects of antibiotics, particularly combinations of rifampicin and tetracycline, on the reproductive capacity of Ae. albopictus females.

Key words: Aedes albopictus, ovary, rifampicin, tetracycline, Wolbachia

Introduction
Mosquitoes of the genus Aedes transmit infectious diseases including dengue fever, chikungunya, and filariasis (Gubler 1998). Among them, the Asian tiger mosquito Aedes albopictus is an invasive vector species that is an emerging public health concern worldwide (Bonizzoni, Gasperi, Chen, & James 2013). In the Republic of Korea, Ae. albopictus could be the vector for various viruses (Chang, Kim, Ha, et al. 2018). To control mosquito populations, various strategies have been used including insecticides. However, increased resistance to insecticides has prompted efforts to develop alternative methods. A new approach using a symbiotic bacteria including Wolbachia has been tested and used in several countries (Hegde, Rasgon, & Hughes 2015; Iturbe-Ormaetxe, Walker, & Sl 2011). However, there are many kinds of antibiotics in the mosquito habitats. Antibiotics have been used to remove bacteria including Wolbachia from the mosquito (Endersby-Harshman, Axford, & Hoffmann 2019; Nazir, Khan, & Qiu 2019; Scates, O’neal, & Anderson 2019). Mosquitoes could be exposed to antibiotics in various ways. In the field, mosquito may hatch in water contaminated by antibiotics and could be exposed throughout their development. (Endersby-Harshman et al. 2019). In another case scenario, when a mosquito bites the host, antibiotics can enter the mosquito body (Gendrin et al. 2015; Thayer 1973). In laboratory conditions, antibiotics have been used to remove the microbes from mosquito gut (Ballard & Melvin 2007; Mousson et al. 2010). Elimination of microbes from their host by antibiotics has been applied to examine
the role of microbes (Ballard & Melvin 2007, Mousson et al. 2010).

Antibiotics affect mosquitoes in various ways. They affect the lysis of red blood cells, delay protein digestion and reduce egg production in female mosquitoes (Gaio Ade et al. 2011). They are also known to suppress protein biosynthesis and to produce toxic effects in the host (Arenz & Wilson 2016). In addition, antibiotics affect the mosquito by interfering with the microbe-induced cytoplasmic incompatibility (CI) during reproduction (Endersby-Harshman et al. 2019). For example, the antibiotics tetracycline hydrochloride and chloramphenicol make the cross Aedes polynesiensis male × Aedes kesseli female incompatible (Trpis, Perrone, Reissig, & Parker 1981). The use of antibiotics is required for Wolbachia removal to avoid genetic depression in the outbreeding procedures (Calvitti, Moretti, Skidmore, & Dobson 2012). In addition, the cell structure of the ovary could be changed by feeding to formation of matured eggs (Greif 2016). Therefore, exposure to antibiotics might have an impact on the reproductive organ of mosquitoes. However, very little is known about the effect of antibiotics in the ovary and reproductive capacity of Aedes albopictus.

In this study, we investigated the effect of antibiotics (tetracycline and combinations of rifampicin) on reproductive capacity of Aedes albopictus. To compare the effects of antibiotics, the hatching rate and fertility were investigated. Additionally, the morphology of follicles and nurse cells of the ovary following treatment with antibiotics were confirmed histologically by hematoxylin and eosin (H&E) staining. After treatment with tetracycline and combinations of rifampicin with tetracycline, the distribution of reactive oxygen species (ROS) was visualized in the ovaries of Aedes albopictus. Our experimental results reveal the influence of antibiotics on the ovaries of Aedes albopictus.

Materials and Methods

Mosquito rearing

Aedes albopictus (Skuse) were collected from Cheongju-si, South Korea, and screened out following identification keys. They were reared and adapted under standard laboratory conditions. Mosquitoes were maintained on a 10% sugar solution at 27 ± 1°C and 65–75% humidity with a 12-h:12-h light/dark cycle. Eggs were hatched in water and larvae were reared in a 2 L plastic container. After pupation, the mosquitoes were transferred to insect rearing cages. Eggs were collected on filter paper with distilled water. This study was conducted strictly in accordance to the guidelines and recommendations provided in the ‘Guide for the Care and Use of Laboratory Animals’ of the National Institutes of Health (Korea NIH, Cheongju, Republic of Korea).

Antibiotic treatment to eliminate Wolbachia

Aedes albopictus mosquito samples were collected from the 10th generation (F10) under laboratory conditions. They were treated with tetracycline and rifampicin to obtain a Wolbachia-free strain as described previously (Mousson et al. 2010). First instar larvae and pupae were treated with 10 mg/L tetracycline (Sigma Aldrich, St. Louis, MO, USA) in the F10 generation. Subsequently, 20 mg/L tetracycline was treated in larval and pupal stages of F11 generation. Additionally, 40 mg/L tetracycline was used to treat the F12 and F13 generations during the larval and pupal stages. After the last larval and pupal treatment, only the F13 generation was treated with rifampicin (2.5 g/L) (Sigma Aldrich) dissolved in 10% sucrose solution. In this study, 10 mg/L of tetracycline-treated F10 generation and 40 mg/L of tetracycline and 2.5 g/L rifampicin-treated F13 generation were investigated for comparison of single and combination treatment. These treatment groups were compared with the untreated group.

Egg hatching and bleaching

After treatment with 10 or 40 mg/L tetracycline and 2.5 g/L rifampicin, blood-fed females laid eggs, which were used for egg hatching. A total of 100 eggs from each group on a small piece of filter paper were placed in a 400-mL plastic container. The mosquito eggs were flooded with a 0.25 g/L yeast solution in distilled water. The hatched larvae were counted for up to 24 h. All experiments were performed in triplicate. The eggs were bleached with 50% sodium hypochlorite solution containing approximately 6% chlorine for 20 min to determine whether the eggs were embryonated. The bleached eggs were viewed and counted under a microscope (Olympus, CKX53 culture microscope, Tokyo, Japan).

Histopathological examination

Mosquitoes were fixed in 10% formalin overnight at 4°C and 0.25% Triton X-100 at room temperature for 15 min. Fixed mosquitoes were washed three times with phosphate-buffered saline (PBS) and embedded in paraffin. Using a standard protocol (Kpclab, Gwangju, Gyeonggi, South Korea), the ovary section slices were stained with haematoxylin and eosin. The stained ovary sections were observed under a CKX53 Olympus inverted microscope with a 100 × or 400 × objective lens.

Detection of reactive oxygen species

For ROS detection, the ovary samples were dissected from mosquitoes anaesthetized at 4°C using a needle-like probe and forceps. Subsequently, the ovaries were rinsed in PBS with 0.0025% Triton X-100. The rinsed ovaries were stained...
with 30 μM concentration of CM-H₂DCFDA (a chloromethyl derivative of H₂DCFDA (5-(and-6)-chloromethyl-2', 7'-dichlorodihydrofluorescein diacetate). Subsequently, the ovaries were incubated for 5–15 min at room temperature (in dark conditions) using a 30 μM solution of CM-H₂DCFDA in PBS. After rinsing in PBS, the intracellular ROS generation in the ovaries were observed with a fluorescent microscope. Fluorescence was visualized at excitation and emission wavelengths of 490 and 530 nm, respectively. The CM-H₂DCFDA is a fluorescent probe that reacts with intracellular ROS (Eruslanov & Kusmartsev 2010) and reveals the distribution of ROS within follicles.

### RNA isolation and reverse transcription-polymerase chain reaction (RT-PCR)

From the dissected ovaries, RNA was isolated using an RNA extraction kit (Qiagen, Hilden, Germany). RT-PCR was conducted using a DiaStar 2 × OneStep RT-PCR premix kit (SolGent, Daejeon, South Korea). Wolbachia surface protein (WSP) primers were used to confirm the presence of Wolbachia (Zhou, Rousseau, & O’neil 1998). β-Actin was also detected using previously described primers (Hirakawa et al. 2016). TLR2 and Myd88 primers for Ae. albopictus were designed using the online Primer3 web-based interface (http://frodo.wi.mit.edu/primer3/) (Table 1). RT-PCR was performed under the following conditions: an initial step at 50°C for 30 min for reverse transcription and 95°C for 15 min for denaturation, followed by 30 cycles of 45 s at 95°C, 52 s at 52°C, and 45 s at 72°C, with a final extension step at 72°C for 10 min. To determine the band intensity, relative expression changes were calculated between the intensity of samples normalized to the β-actin housekeeping gene using ImageLab software version 5.2 (Bio-Rad, Hercules, CA, USA).

#### Statistical analysis

Mean values were calculated in triplicate. The results are expressed as the mean ± standard deviation. All data were statistically analyzed using the t-test (P value) in Sigma Plot, version 12.5 (Systat Software, Inc., San Jose, CA, USA). Significance values of P < 0.05 were considered to indicate statistical significance.

### Results

#### Effects of antibiotics on egg hatching

To investigate the effect of antibiotics on egg hatching, 10 mg/L tetracycline or 40 mg/L tetracycline with 2.5 g/L rifampicin were treated. The average hatching rates were significantly affected by antibiotic treatment. The average hatching rates were approximately 80.0 ± 18.3% and 69.6 ± 19.2% in the untreated group and 10 mg/L tetracycline-treated group, respectively. In the group treated with 40 mg/L tetracycline with 2.5 g/L rifampicin, the average hatching rate was 7.0 ± 14.0% (Fig. 1). Therefore, when Ae. albopictus were treated with 10 mg/L tetracycline and 40 mg/L tetracycline with 2.5 g/L rifampicin, a respective decline in egg hatch by 13.0% and 91.2%, was observed compared to untreated group. This infers that the egg hatching rate was significantly impaired in the combination antibiotics treatment group when compared with untreated samples and treatment with 10 mg/L tetracycline (P < 0.05).

#### Effects of antibiotics on egg fertility

To determine the effect of antibiotics on fertility and infertility, the eggs were bleached and embryonated and unembryonated eggs were distinguished. Bleach digestion removes the egg chorion while leaving the serosal cuticle intact to observe the formation of embryogenesis. Figure 2A shows the unbleached eggs which were not treated with bleach solution and antibiotics. After bleaching the eggs with sodium hypochlorite solution, the unembryonated eggs (Fig. 2B) and embryonated eggs (Fig. 2C) were visualized. The percentage of embryonated and unembryonated eggs was counted in antibiotics-treated and untreated groups. The percentages of

| Gene   | Primer sequence 50-3′   | PCR product size (bp) |
|--------|-------------------------|-----------------------|
| WSP    | F: TGG TCC AAT AAG TGA TGA AGA AAC; R: AAA AAT TAA ACG CTA CTC CA | 220 |
| TLR2   | F: CGC TTT TGT GAA CTT GGA GAC; R: GGT TAG GGA CGG TGG AAA TAA | 157 |
| TLR6   | F: GCG AGT TGG GAG GAT ACA GTT; R: AGA GGT CCA GGT TTA CGA ACG | 138 |
| Myd88  | F: AAA GAT GAC ACA CGG CAG AAG; R: CGA AAT AAC AAC CAC CAG TCG | 219 |
| Cactus | F: GAT AGA CTC GGG CTT TCA TTC; R: GGG GCT GCT TCT GGT TTC | 137 |
| β-actin| F: AGA TCA TGT TCG AGA CCT TC; R: TCA GGA TCT TCA TCA GGT AA | 209 |
embryonated eggs were 89.43 ± 9.09%, 97.48 ± 2.39%, and 62.66 ± 3.92% in the untreated group, 10 mg/L tetracycline-treated group, and 40 mg/L tetracycline and 2.5 g/L rifampicin-treated group, respectively (Fig. 2D).

When *Ae. albopictus* were treated with 40 mg/L tetracycline with 2.5 g/L rifampicin, a respective decline in percentage of embryonated eggs by 30.0%, was observed compared to untreated group. This result indicates that the egg fertility
was significantly declined in the combination antibiotics treatment group when compared with untreated samples and treatment with 10 mg/L tetracycline ($P < 0.05$).

**Histopathological effect of antibiotics in the ovary**

The normal follicle forms the vitelline membrane between the oocyte and epithelial cells (Fig. 3A and B). The epithelial cell layer was thickened in the group treated with 40 mg/L tetracycline + 2.5 g/L rifampicin (Fig. 3C and D). Further, the boundary between the oocyte and nurse cells was obscured compared to the untreated group. In the oocyte, degradation of the yolk granules was observed (Fig. 3C and D). A light region at the periphery of the oocyte was more prominent within the epithelial follicles in the 40 mg/L tetracycline with 2.5 g/L rifampicin-treated group compared to the untreated group. Hence, the combinations of antibiotics induced histopathological damages in the ovary of *Ae. albopictus*.

**Detection of ROS in the mosquito ovary**

Figure 4A and C show optical images of untreated and 40 mg/L tetracycline with 2.5 g/L rifampicin-treated ovaries. The follicles showed a weak fluorescence signal in the untreated group (Fig. 4B). In contrast, a strong signal was observed in the oocyte nucleus and nurse cell nucleus in the 40 mg/L tetracycline with 2.5 g/L rifampicin-treated group compared to the untreated group. The signal from the oocyte cytoplasm was much weaker compared to that in the nucleus (Fig. 4D).

**Ovary-specific expression of Toll like receptors in the ovaries**

Following exposure to antibiotics, *Wolbachia* surface protein (WSP) expression was decreased in a dose-dependent manner (Fig. 5). In the 10 mg/L tetracycline-treated group, TLR2 and Myd88 expression was up-regulated 1.5 and 6.1 times, compared to in the untreated group, respectively. TLR2, TLR6, and Myd88 expression was significantly up-regulated 1.2, 3.3 and 8.5 times in 40 mg/L rifampicin-treated ovaries compared to in the untreated group, respectively. In contrast, Cactus expression was downregulated about 0.7 times in the 40 mg/L tetracycline and 2.5 g/L rifampicin-treated group, compared with the 10 mg/L tetracycline-treated group and untreated group (Fig. 5).

**Discussion**

Mosquitoes have been able to adapt to various habitats and are considered vectors for viruses causing clinical manifestations in humans (Ricci et al. 2012). With the environmental contamination by antibiotics ever increasing, mosquitoes are at a greater risk to be exposed (Endersby-Harshman et al. 2019; Gaio Ade et al. 2011). In most cases, mosquitoes such as *Ae.*
Aedes aegypti get exposed to greater antibiotic concentrations when they inhabit drains or underground water resources than when they breed in containers filled by rain or drinking water (Endersby-Harshman et al. 2019). Antibiotics such as tetracycline and rifampicin can be particularly devastating in their mode of action. Tetracycline is a broad-spectrum antibiotic for Gram-positive and Gram-negative bacteria, chlamydiae, mycoplasma, protozoan parasites, or rickettsiae (Ballard & Melvin 2007). It can inhibit mitochondrial protein synthesis and delay the oviposition in insect (Kurlovs, Li, Cheng, & Zhong 2014; Zeh, Bonilla, Adrian, Mesfin, & Zeh 2012). Rifampicin is a lipid-soluble antibiotic that can penetrate membranes and concentrate in cells (Dhillon & Mitchison 1989). It influences bacterial diversity and richness that can be incompletely recovered even after discontinuing the antibiotic (Rosas et al. 2018). The presence of antibiotics including tetracycline in the environment has been
investigated in previous study. Tetracycline concentrations in reported field maxima in mosquito container habitats ranged from 0.04 to 0.97 μg/L (Curtis, Matzen, Neira Oviedo, et al. 2015). Its level in animal waste holding ponds is 1,000 μg/L (Campagnolo, Johnson, Karpati, et al. 2002). Tetracycline at a concentration of 1.0 to 5.0 × 10^6 μg/L was required to clear mosquitoes of Wolbachia infection in the laboratory (Li, Floate, Fields, & Pang 2014). This is significant as Wolbachia infected mosquitoes have been used in the dengue control international programs wherein these are introduced into endemic regions to replace the resident populations by means of CI (McMeniman et al. 2009; Walker, Johnson, Moreira, et al. 2011; Flores & O’Neill 2018). Mosquito control using Wolbachia could be affected by antibiotic pollution in the environment.

However, the chemical toxicity of antibiotics towards reproductive physiology of mosquitoes has still not been fully explored. In this study, we detailed the effects of the antibiotics tetracycline and rifampicin on egg hatching and fertility. Antibiotic treatment reduced the hatching of eggs with a lesser number of embryonated eggs. Hence, the results suggest a direct correlation relating to the influence of antibiotics on the reproductive capacity of mosquitoes. The reduced hatching rate and fertility in mosquitoes may be ascribed to the inhibition of amino acid absorption (Chopra & Roberts 2001; Thayer 1973). A total of ten amino acids, including arginine, valine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and histidine, have been found to be essential for egg production and larval development (Dimond, Lea, Hahnert, & Delong 1956). This substantiates the requirement of amino acid absorption towards reproductive capacity in mosquitoes. Another reason for reduced reproductive capacity in mosquitoes is the clearance of bacteria through antibiotics exposure. Especially, Wolbachia are intracellular bacteria and are maternally inherited as an infection by many arthropod species (Dobson et al. 1999). Wolbachia effects reproductive alterations, such as feminization, parthenogenesis, male killing, and CI in arthropods (Noda, Miyoshi, & Koizumi 2002). Interactions between Wolbachia and the host can influence fecundity, immune response, stress response, and metabolite levels (Brownlie et al. 2009; Caragata et al. 2013; Dutra et al. 2017). In this study, we found dose-dependent decrease in the expression of WSP that in turn stimulates increased transcription of immune genes in mosquitoes. In addition, Wolbachia downregulates apoptosis in the developing ovaries. Therefore, elimination of bacteria leads to the apoptosis of ovarian cells (Pannebakker, Loppin, Elemans, Humblot, & Vavre 2007; Werren, Baldo, & Clark 2008). Our results corroborate the earlier findings showing reduced hatching rates, fertility, and ovarian damage following antibiotics treatment with Wolbachia inhibition.

Treatment with antibiotics may affect the innate immune system of mosquitoes. The major immune signaling cascades in mosquito are Toll, immune deficiency (IMD), and Janus kinase/signal transducers and activators of transcription (JAK–STAT) pathways. RNA interference (RNAi) pathway is not a classical innate immune pathway, but plays an important role in antiviral defense (Sim, Jupatanakul, & Dimopoulos 2014). In a previous study, the innate immune responses to Wolbachia in Brugia malayi and Onchocerca volvulus was found dependent on Toll like receptors (TLRs), such as TLR2, TLR6, and Myd88, but not TLR4 (Hise, Daehnel, Gillette-Ferguson, et al. 2007). In addition, Wolbachia induced ROS-dependent activation of the Toll pathway in Ae. aegypti (Pan et al. 2012). In our examination of the Toll pathway-regulated genes using RT-PCR, we found that the treatment of a high dose (40 mg/L) of tetracycline with rifampicin induces TLR2, TLR6, and Myd88 mRNA expression in the ovaries. Alternatively, the expression of Cactus was downregulated in the ovaries. The Toll signaling is triggered by the Toll receptors that are activated by ligands. Adaptor proteins such as Myd88 lead to the phosphorylation and degradation of Cactus. Subsequently, Cactus degradation activates the transcription of Toll pathway-regulated genes (Sim et al. 2014). From our results, it is understood that the Toll pathway may be directly and indirectly activated by antibiotics in the ovary of mosquitoes. However, further detailing of reproductive physiology of female mosquitoes is needed to explain the detailed effects of broad-spectrum antibiotics such as tetracycline and rifampicin.

In conclusion, our study provides an increased understanding of the ovarian physiology in Ae. albopictus mosquitoes following treatment with antibiotics such as tetracycline and rifampicin. The hatching rate, fertility rate, histopathological changes, and altered TLR expression triggered by antibiotics were investigated. To the best of our knowledge, this is the first study to report on the effect of antibiotics in the ovary and reproductive capacity of Ae. albopictus. Given the findings of this study, the level of antibiotics in rearing mosquitoes will assist in understanding the reproductive capacity of mosquitoes involved in the mosquito control.

Competing financial interests
The authors declare no competing financial interests.

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References

Arenz S, Wilson DN (2016) Bacterial Protein Synthesis as a Target for Antibiotic Inhibition. Cold Spring Harbor Perspectives in Medicine 6: a025361.

Ballard JW, Melvin RG (2007) Tetracycline treatment influences mitochondrial metabolism and mtDNA density two generations after treatment in Drosophila. Insect Molecular Biology 16: 799–802.

Bonizzoni M, Gasperi G, Chen X et al. (2013) The invasive mosquito species Aedes albopictus: current knowledge and future perspectives. Trends in Parasitology 29: 460–468.

Brownlie JC, Cass BN, Riegler M et al. (2009) Evidence for metabolic provisioning by a common invertebrate endosymbiont, Wolbachia pipiensis, during periods of nutritional stress. PLoS Pathogens 5: e1000368.

Calvitti M, Moretti R, Skidmore AR et al. (2012) Wolbachia strain wPip yields a pattern of cytoplasmic incompatibility enhancing a Wolbachia-bias suppression strategy against the disease vector Aedes albopictus. Parasites & Vectors 5: 254.

Campagnolo ER, Johnson KR, Karpati A et al. (2002) Antimicrobial residues in animal waste and water resources proximal to large-scale swine and poultry feeding operations. Science of the Total Environment 299: 89–95.

Caragata EP, Rances E, Hedges LM et al. (2013) Dietary cholesterol modulates pathogen blocking by Wolbachia. PLoS Pathogens 9: e1003459.

Chang KS, Kim GH, Ha YR et al. (2018) Monitoring and Control of Aedes albopictus, a Vector of Zika Virus, Near Residences of Imported Zika Virus Patients during 2016 in South Korea. The American Journal of Tropical Medicine and Hygiene 98: 166–172.

Chopra I, Roberts MJMBr (2001) Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiology and Molecular Biology Reviews 65: 232–260.

Curtis Z, Matzen K, Neira Oviedo M et al. (2015) Assessment of the Impact of Potential Tetracycline Exposure on the Phenotype of Aedes aegypti OX513A: Implications for Field Use. PLoS Neglected Tropical Diseases 9: e003999.

Dhillon J, Mitchison DA (1989) Activity and penetration of antituberculosis drugs in mouse peritoneal macrophages infected with Mycobacterium microti OV254. Antimicrobial Agents and Chemotherapy 33: 1255–1259.

Dimond J, Lea A, Hahnert W et al. (1956) The amino acids required for egg production in Aedes aegypti. The Canadian Entomologist 88: 57–62.

Dobson SL, Bourtzis K, Braig HR et al. (1999) Wolbachia infections are distributed throughout insect somatic and germ line tissues. Insect Biochemistry and Molecular Biology 29: 153–160.

Dutra HLC, Rodrigues SL, Mansur SB et al. (2017) Development and physiological effects of an artificial diet for Wolbachia-infected Aedes aegypti. Scientific Reports 7: 15687.

Endersby-Harshman NM, Axford JK, Hoffmann AA (2019) Environmental Concentrations of Antibiotics May Diminish Wolbachia infections in Aedes aegypti (Diptera: Culicidae). Journal of Medical Entomology 56: 1078–1086.

Eruslanov E, Kusmartsev S (2010) Identification of ROS using oxidized DCFDA and flow-cytometry. Methods in Molecular Biology 594: 57–72.

Flores HA, O’Neill SL (2018) Controlling vector-borne diseases by releasing modified mosquitoes. Nature Reviews Microbiology 16: 508–518.

Gaio Ade O, Gusmao DS, Santos AV et al. (2011) Contribution of midgut bacteria to blood digestion and egg production in Aedes aegypti (diptera: culicidae) (L.). Parasites and Vectors 4: 105.

Gendrin M, Rodgers FH, Yerbanga RS et al. (2015) Antibiotics in ingested human blood affect the mosquito microbiota and capacity to transmit malaria. Nature Communications 6: 5921.

Greif MM (2016) Studies on ovaries of mosquitoes using light and scanning microscopy. International Journal of Mosquito Research 3: 47–50.

Gubler DJ (1998) Resurgent vector-borne diseases as a global health problem. Emerging Infectious Diseases 4: 442–450.

Hegele S, Rasgon JL, Hughes GL (2015) The microbiome modulates arbovirus transmission in mosquitoes. Current Opinion in Virology 15: 97–102.

Hise AG, Daehnel K, Gillette-Ferguson I et al. (2007) Innate immune responses to endosymbiotic Wolbachia bacteria in Brugia malayi and Onchocerca volvulus are dependent on TLR2, TLR6, MyD88, and Mal, but not TLR4, TRIF, or TRAM. The Journal of Immunology 178: 1068–1076.

Iturbe-Ormaetxe I, Walker T, SI ON (2011) Wolbachia and the biological control of mosquito-borne disease. EMBO Reports 12: 508–518.

Kurlov AH, Li J, Cheng D et al. (2014) Ixodes pacificus ticks maintain embryogenesis and egg hatching after antibiotic treatment of Rickettsia endosymbiont. PLoS ONE 9: e104815.

Li Y-Y, Floate K, Fields P et al. (2014) Review of treatment methods to remove Wolbachia bacteria from arthropods. Symbiosis 62: 1–15.

Memeniman CJ, Lane RV, Cass BN et al. (2009) Stable introduction of a life-shortening Wolbachia infection into the mosquito Aedes aegypti. Science 323: 141–144.

Mousson L, Martin E, Zouache K et al. (2010) Wolbachia modulates Chikungunya replication in Aedes albopictus. Molecular Ecology 19: 1953–1964.

Nazir T, Khan S, Qiu D (2019) Biological Control of Insect Pest. Pests-Insects, Management, Control. IntechOpen.

Noda H, Miyoshi T, Koizumi Y (2002) In vitro cultivation of Wolbachia in insect and mammalian cell lines. In Vitro Cellular & Developmental Biology - Animal 38: 423–427.

Pan X, Zhou G, Wu J et al. (2012) Wolbachia induces reactive oxygen species (ROS)-dependent activation of the Toll pathway to control dengue virus in the mosquito Aedes aegypti. Proceedings of the National Academy of Sciences of the United States of America 109: E23–E31.
Pannebakker BA, Loppin B, Elemans CP et al. (2007) Parasitic inhibition of cell death facilitates symbiosis. Proceedings of the National Academy of Sciences of the United States of America 104: 213–215.

Park CH, Lim H, Kim H et al. (2016) High prevalence of Wolbachia infection in Korean populations of Aedes albopictus (Diptera: Culicidae). Journal of Asia-Pacific Entomology 19: 191–194.

Ricci I, Damiani C, Capone A et al. (2012) Mosquito/microbiota interactions: from complex relationships to biotechnological perspectives. Current Opinion in Microbiology 15: 278–284.

Rifampicin treatment of Blattella germanica evidences a fecal transmission route of their gut microbiota. MS Microbiology Ecology 94.

Scates SS, O’neal ST, Anderson TD (2019) Bacteria-mediated modification of insecticide toxicity in the yellow fever mosquito, Aedes aegypti. Pesticide Biochemistry and Physiology 161: 77–85.

Sim S, Jupatanakul N, Dimopoulos G (2014) Mosquito immunity against arboviruses. Viruses 6: 4479–4504.

Thayer DW (1973) Effect of antibiotics and antimalarials on free amino acids of Aedes aegypti. Journal of Medical Entomology 10: 57–62.

Trpis M, Perrone JB, Reissig M et al. (1981) Control of cytoplasmic incompatibility in the Aedes scutellaris complex: Incompatible crosses become compatible by treatment of larvae with heat or antibiotics. Journal of Heredity 72: 313–317.

Walker T, Johnson PH, Moreira LA et al. (2011) The wMel Wolbachia strain blocks dengue and invades caged Aedes aegypti populations. Nature 476: 450–453.

Werren JH, Baldo L, Clark ME (2008) Wolbachia: master manipulators of invertebrate biology. Nature Reviews Microbiology 6: 741–751.

Zeh JA, Bonilla MM, Adrian AJ et al. (2012) From father to son: transgenerational effect of tetracycline on sperm viability. Scientific Reports 2: 375.

Zhou W, Rousset F, O’neil S (1998) Phylogeny and PCR-based classification of Wolbachia strains using wsp gene sequences. Proceedings of the Royal Society B: Biological Sciences 265: 509–515.