

## Research Article

# Bacteria Load Determination of the Intestinal Microbiota and Identification of *Spiroplasma* and *Wolbachia* in *Anopheles gambiae*

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The gut microbiota of mosquitoes is composed of a range of microorganisms. Among its microorganisms, some affect the vectorial capacity of mosquitoes. The aim of this study was to characterize some bacteria of the intestinal microbiota in *Anopheles gambiae* (*An. gambiae*) females, a major vector of malaria transmission in Benin. The symbiote bacteria of the microbiota of female laboratory *An. gambiae* and female wild *An. gambiae* were identified by the culture method. The count was done on media plate count agar (PCA), and subsequently, the bacterial load was calculated. Comparison of batches bacterial load was carried out with the variance analysis test (ANOVA). Finally, polymerase chain reaction (PCR) was performed to investigate the presence of a few bacterial genera influencing the vector capacity of *An. gambiae*. The study found that the microbiota of female *An. gambiae* is home to the bacteria belonging to the *Staphylococcus*, *Enterobacteriaceae*, and other unidentified bacterial gene regardless of its nature and condition. Similarly, there was no statistically significant difference between the bacterial load of the laboratory and wild mosquitoes depending on the parous and gorged states; on the other hand, there was a significant difference between the bacterial loads of the laboratory and wild mosquitoes according to the nulliparous and nongorged states. The search for a few bacterial genera influencing the vector capacity of female *An. gambiae* has been negative for *Spiroplasma* bacteria regardless of its nature and condition. PCR revealed the presence of *Wolbachia* bacteria for only gorged Kisumu sensitive *An. gambiae*. *Wolbachia*'s presence at *An. gambiae* suggests that this type of bacteria could be used to develop new effective and sustainable approaches in the vector control.

## 1. Introduction

Malaria remains a public health problem in many developing countries, particularly in Sub-Saharan Africa [1]. The dynamics of malaria transmission depend on certain aspects of the physiology and ecology of their vectors, mosquitoes. To break the cycle of the disease transmission, vector control

is the most common strategy in endemic countries [2]. However, the heavy use of insecticides to carry out this strategy modifies the trophic behavior of the vectors [3]. This has the consequence of negatively impacting the effort to reduce the burden of the disease due to malaria. As a result, new strategies are desperately needed to control mosquito populations or their ability to transmit parasites. One of the

promising strategies to reduce vector transmission is to involve bacterial symbionts which have the potential to decrease the vectorial capacity of their hosts [4]. Indeed, these symbionts are maintained by maternal transmission and can therefore spread within populations. With these characteristics, the symbionts appear to be a much more sustainable and cost-effective strategy for controlling the transmission of vector-borne diseases such as malaria [5]. These microorganisms, including bacteria, viruses, fungi, protozoa, nematodes, and mites, are more or less stable in the exoskeleton, intestine, hemocoel, and/or in mosquito cells [6].

To advance the prospect of a symbiont-based malaria control strategy, it will be important to continue to identify, generate, and study a wide range of anopheles-symbiont systems. The mosquito microbiota is of particular interest because of its influence on certain factors determining the mosquito's ability to transmit pathogens such as immunity, longevity, fertility, and metabolism [6]. They can be pathogens, commensals, or mutualists and rely on vertical or horizontal transmission [7]. In addition, the symbiont bacteria are functionally diverse, exhibiting a wide range of infection and transmission strategies. The mosquito gut is naturally inhabited by a community of bacteria that can disrupt the development of human parasites such as *Plasmodium* [8]. In some mosquitoes, as in many species of arthropods, two major taxa of facultative endosymbionts have been identified, *Wolbachia* and *Spiroplasma*. Since these bacteria are transmitted vertically from the mother, they have acquired the particularity of modifying the reproduction of their host, thus increasing their spread, by mechanisms that vary according to the species of arthropod and the bacterial strain [6].

*Wolbachia* has recently been reported in low percentages in some populations of *Anopheles coluzzi* and *Anopheles gambiae* [9]. *Wolbachia* appears to induce cytoplasmic incompatibility since spermatozoa from the infected males could not fertilize oocytes from the uninfected females. This promotes the reproduction of infected females over uninfected females in a population. These bacteria can also increase the proportion of females in a population by the induction of parthenogenesis, by male-specific lethality during development or by feminization of males [10]. In addition, *Spiroplasmas* are "specialists" in arthropods, and all known species have some form of interaction with this clade [11], and they can confer a variety of resistant insect hosts on a range of eukaryotic parasites, including nematodes, parasitoids, and pathogenic fungi [12, 13]. Therefore, they are a good candidate for a symbiont which could be useful for the control of *Plasmodium*.

Some studies on the microbiome of anopheles have identified *Spiroplasma* from pathogenic clades [14, 15]. However, in Benin, we have very little data on the bacterial symbionts of the major vector of malaria transmission. It is in this dynamic that fits this study that aim to investigate this microbial diversity, focusing on some bacteria, of the intestinal microbiota in female *An. gambiae*.

## 2. Materials and Methods

**2.1. Sample Collection.** Batches of mosquitoes (wild or reared) were made for this study. The reared mosquitoes' batches (Kisumu and Kdr) come from the Centre de Recherche en Entomologie de Cotonou (CREC) insectarium. The parity parameters (parous and nulliparous) and nutritional status (gorged and not gorged) were considered for the constitution of the batches. Thus, for Kisumu's and Kdr, four batches (parous, nulliparous, gorged, and not gorged) were made up. Each batch of mosquitoes consisted of five randomly selected individuals from the same clutch and were stored in a 1.5 ml Eppendorf tube at 4°C for later microbiological analysis.

For wild mosquitoes, they were captured by the pyrethrum spray catches (PSC) technique as described in the Manual of Malaria Entomology and Vector Control [16]. Before performing the PSC, all large pieces of furniture were removed, and the floor was covered with white bed sheets. Insecticide was first sprayed from the outside of the house onto the windows and the doors before spraying on the inside of the house. All doors and windows remained closed for about 10 min to induce mosquito knockdown [17]. After exposition time (10 minutes), mosquitoes knocked down were collected, and a preliminary sorting was done on the basis of macroscopic observation. The identification of the species *An. gambiae* was made according to the taxonomic key [17, 18] using the stereo microscope ez4 w binocular (Leica, Germany). The wild mosquitoes were also divided into four batches (parous, nulliparous, gorged, and not gorged) in different 1.5 ml Eppendorf tubes.

**2.2. Microbial Analysis.** Five mosquitoes were randomly selected from each category (parous, nulliparous, gorged, and not gorged), from each batch of *An. gambiae* female (Kisumu, Kdr, and wild). Each mosquito abdomen was separated from the thorax using a sterile scalpel for each sample. The abdomens of selected mosquitoes from the same batches were rinsed in sterile water and then was sterilized in 70% ethanol (1 minute). Thus, each abdomen batches were crushed [19] into 1.5 ml Eppendorf tube containing 500 µl of sterile saline solution to have the stock solution. Finally, 25 µl was taken from each stock solution in order to carry out successive decimal dilutions up to 10<sup>-7</sup>.

Eosin-methylene blue (EMB), Muller Hinton (MH), and Chapman agars were used for seeding the stock solutions, in tight streaks on the first half of the agar, and wide streaks on the second half so as to obtain the isolated colonies. All the inoculated dishes were incubated at 30°C overnight. A first layer of the plate count agar (PCA) was poured into the kneaded dishes, then 25 µl of each successive dilution serving as an inoculum was transferred into the precast dishes, and then homogenized correctly. The second layer was poured as soon as the homogenate started to solidify. The cast boxes were placed in an oven at 37°C for 72 hours. The inoculated boxes were read after 72 hours.

Since the PCA agar is a nutrient medium, it was used to enumerate the total aerobic-mesophilic flora of the

abdominal microbiota of each mosquito. Only dishes with a number of colonies greater than or equal to 30 and less than 300 are taken into account for the enumeration. To determine the bacterial load of the microbiota of each mosquito, the standard formula used is as follows: bacterial load = number of colonies counted  $\times$  seeded volume  $\times$  dilution factor [20].

### 2.3. Molecular Analysis

**2.3.1. DNA Extraction.** Deoxyribose nucleic acid (DNA) extraction was performed from a pool of mosquitoes using the QIAamp DNA Mini Kit (Quiagen, Germany). The extraction protocol provided by the manufacturer with the kit was strictly followed. Thus, in brief, the abdomens of each mosquito were pooled in a 1.5 ml Eppendorf and filled with 180 (microliter/microliter)  $\mu$ l of ALT buffer + proteinase K. The homogenized mixture was incubated in the oven at 56°C for about 3 hours. After incubation, the mixture was supplemented with 200  $\mu$ l of buffer AL and was homogenized carefully for 15 seconds. Incubated in the oven at 70°C for 10 minutes, the tubes were centrifuged briefly to remove any drops from the lid. After homogenization of the mixture for 15 s, 200  $\mu$ l of ethanol (96%) was added, briefly centrifuged, and the supernatant was transferred to the QIAamp Mini extraction columns for another centrifugation (8000 rpm for 1 minute). The QIAamp Mini Spin extraction column was placed in a new 2 ml collection tube and 500  $\mu$ l of buffer AW1 was added before centrifugation at 8000 rpm for 1 minute, and the collection tube was discarded. The QIAamp Mini extraction column was placed again in a 2 ml collection tube and then 500  $\mu$ l of buffer AW2 was added. The mixture was centrifuged at 14,000 rpm for 3 minutes. The QIAamp Mini Extraction column was placed in a new 2 ml collection tube and was centrifuged at full speed for 1 minute to eliminate the risk of possible carryover of Buffer AW2. To end, 200  $\mu$ l of AE buffer was used for DNA elution in a new 1.5 ml Eppendorf tube and maintained at 4°C.

**2.3.2. Search for Alleles of Genes Use for the Identification of *Spiroplasma* and *Wolbachia*.** For the identification of *Spiroplasma*, the *DnaA* genetic determinant was the target [21]. The primers used for this purpose were *DnaA109F*: 5'-TTAAGAGCAGTTTCAAATCGGG3' and *DnaA246R*: 5'-CAAACAAATGTTATTACTTC-3'. The PCR reactions were performed using the thermal cycler under the following conditions: a cycle of initial denaturation (95°C for 10 minutes), followed by 35 cycles of denaturation (95°C for 30 s), hybridization (55°C for 30 s), and elongation (72°C for 30 s), followed by a final elongation (72°C for 10 minutes).

The molecular identification of *Wolbachia* was made targeting *ftsZ* [22]. For this, the used primers were *ftsZf1* 5'-GTTGTCGCAAATACCGATGC-3' and *ftsZr1* 5'-CTTAAGTAAGCTGGTATATC-3'. In this case, the amplification conditions were the following: initial denaturation (95°C for 10 minutes), followed by 40 cycles of

denaturation (95°C for 30 s), hybridization (60°C for 30 s), and elongation (72°C for 30 s), followed by final elongation (72°C for 10 minutes).

For each gene, the reaction was performed in a 20  $\mu$ l mix containing 10x Eurogentec buffer (2  $\mu$ l), dNTP (0.2  $\mu$ l), 10  $\mu$ M of each primer (1  $\mu$ l), MgCl<sub>2</sub> (1.2  $\mu$ l), *Taq* DNA polymerase (0.2  $\mu$ l), and DNA (3  $\mu$ l). The DNA extracted from mosquitoes was used as a negative control. The amplification products were migrated on a 1.5% agarose gel containing ethidium bromide at 110 V for 30 minutes.

**2.4. Data Analysis.** The data collected was entered into an Excel 2016 spreadsheet and then was analyzed with the Minitab R 18 software. The following steps were taken after the information was collected: (i) checking the normality of the data and the homogeneity of variances, (ii) calculation of means, variances, sum of squares, degree of freedom, mean of squares, and of the value of the probability, and (iii) comparisons of the bacterial load of *An. gambiae* batches. The one-way analysis of the variance and the Fisher test were used to compare the means. The test is considered statistically significant when  $p < 0.05$ .

## 3. Results

**3.1. Bacterial Loads of *Anopheles gambiae* Samples.** *Anopheles gambiae* females had a high bacterial load regardless of the pool and category of mosquito (Figure 1). It was noted that the wild, nongorged female *An. gambiae* had the highest bacterial load, while the wild nulliparous female *An. gambiae* mosquitoes had the lowest bacterial load. The sensitive nongorged Kisumu also had a high bacterial load, whereas gorged Kisumu had the lowest bacterial load. Finally, *An. gambiae* Kdr parous had a high bacterial load as opposed to nulliparous Kisumu which had the lowest bacterial load.

Based on one-way analysis of the variance (ANOVA), the value for the probability (0.095) was greater than 0.05 (Table 1). Therefore, there was no statistically significant difference between the bacterial load of *An. gambiae* mosquitoes from laboratory and wild batches in the parous category at 0.05 level.

Figure 2 shows the difference in bacterial loads, two by two of the different pool of mosquitoes studied in the parous category. This difference in bacterial load is represented on the graph by a confidence interval. It can be seen that all the intervals contain the value zero. Therefore, considering the parous category, there was no statistically significant difference between the bacterial load of mosquitoes from laboratory and wild's (Figure 2). Fisher's test therefore confirms the result obtained from the ANOVA test.

Based on one-way analysis of the variance (ANOVA), the value of the probability of 0.048 was less than 0.05. There was a significant difference between the bacterial load of female *An. gambiae* mosquitoes from laboratory and wilds in the nongorged category ( $p < 0.05$ ) (Table 2).

Figure 3 shows the difference in the bacterial loads two by two of the different pool of mosquitoes studied in the

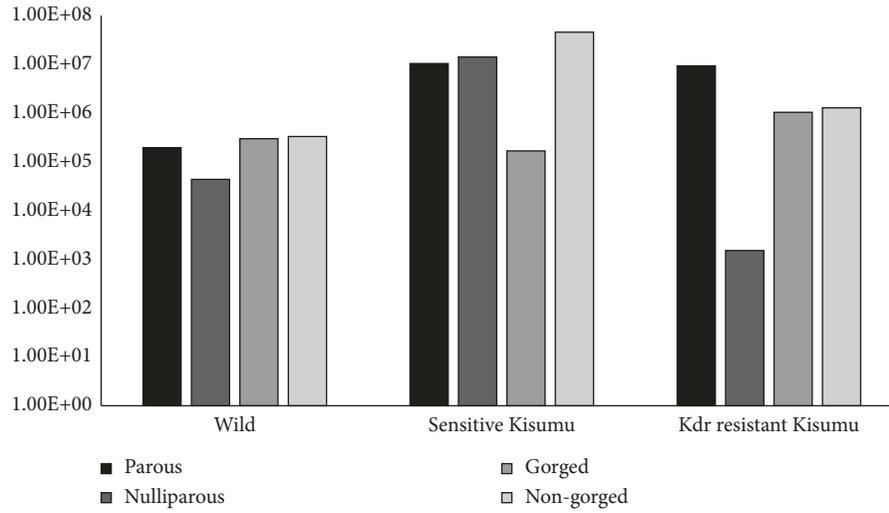


FIGURE 1: Different pool of *Anopheles gambiae* according to their bacterial loads.

TABLE 1: Results of the analysis of the variance test of the different batches of *Anopheles gambiae* mosquito in the parous category.

Source of variations	Sum of squares	Degree of freedom	Average of squares	F	Probability (%)	Critical value for F
Between pools	$1.89 \times 10^{14}$	2	$9.46 \times 10^{13}$	3.574	9.50	5.143
Inside the pools	$1.59 \times 10^{14}$	6	$2.65 \times 10^{13}$			
Total	$3.48 \times 10^{14}$	8				

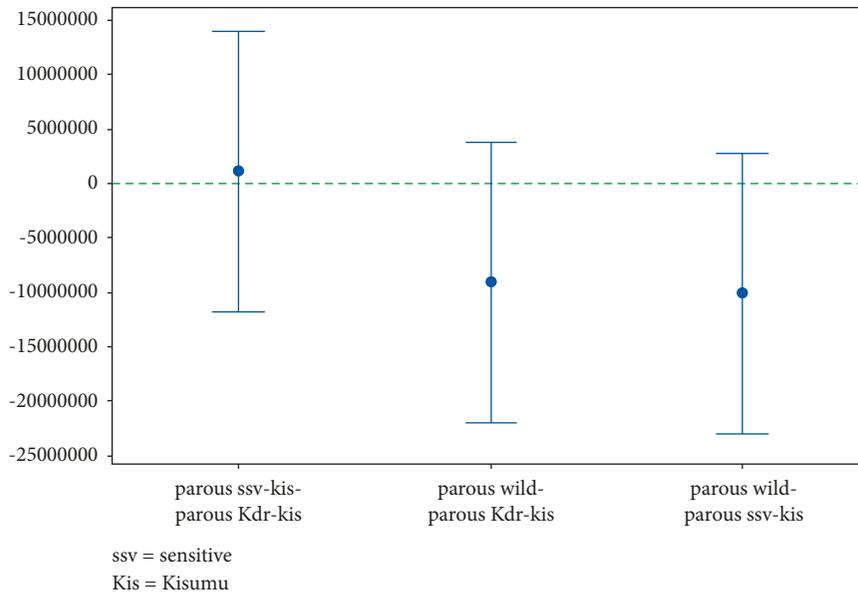


FIGURE 2: Graph resulting from Fisher's test of parous mosquitoes.

TABLE 2: Results of the analysis of the variance test of the different pools of *Anopheles gambiae* in the nongorged category.

Source of variations	Sum of squares	Degree of freedom	Average of squares	F	Probability	Critical value for F
Between pools	$4.06 \times 10^{+15}$	2	$2.03 \times 10^{+15}$	5.27	4.77%	5.14
Inside the pools	$2.31 \times 10^{+15}$	6	$3.81 \times 10^{+14}$			
Total	$6.36 \times 10^{+15}$	8				

parous category. This difference in bacterial loads two by two was represented on the graph by a confidence interval. It can be seen that at least one interval does not contain the value

zero. Consequently, there is a statistically significant difference between the bacterial load of mosquitoes from laboratory and wild batches in the nongorged category

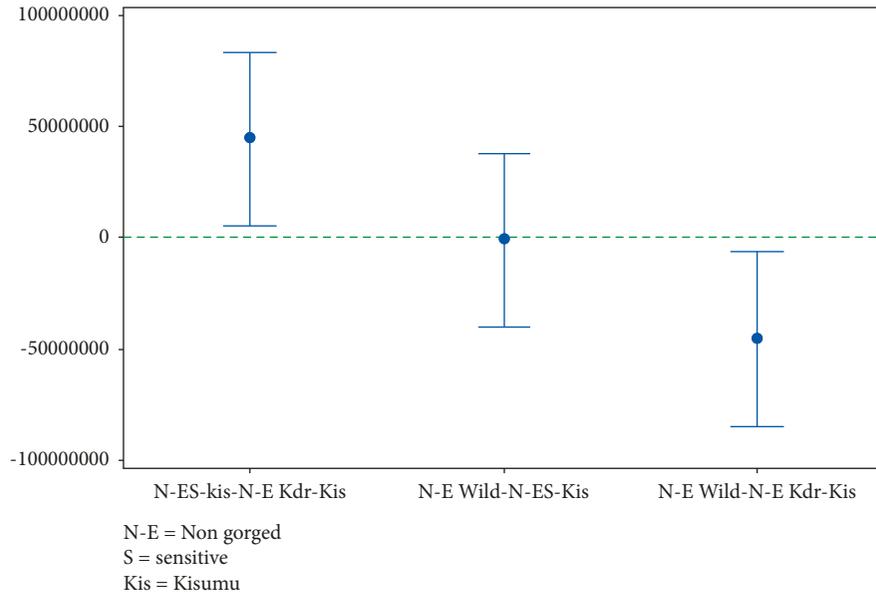


FIGURE 3: Graph results of Fisher's test of nongorged mosquitoes.

(Figure 3). Fisher's test therefore confirms the result obtained from the ANOVA test.

Based on the one-way analysis of variance (ANOVA), there is no statistically significant difference between the bacterial load of mosquitoes from laboratory pools and wilds in the gorged category (Table 3).

Figure 4 shows the difference in bacterial loads of two by two of the different pools of mosquitoes studied in the gorged category. This difference in bacterial loads two by two is represented on the graph by a confidence interval. It can be seen that all the intervals contain the value zero. Consequently, there is no statistically significant difference between the bacterial load of mosquitoes from laboratory and wild batches in the gorged category (Figure 4). Fisher's test therefore confirms the result obtained from the ANOVA test. According to the one-way analysis of variance (ANOVA), there is a statistically significant difference between the bacterial load of mosquitoes from laboratory lots and wilds in the nulliparous category (Table 4).

Figure 5 shows the difference in bacterial loads two by two of the different batches of mosquitoes studied in the nulliparous category. This difference in bacterial loads two by two is represented on the graph by a confidence interval. It can be seen that at least one interval does not contain the value zero. Consequently, there is a statistically significant difference between the bacterial load of mosquitoes from laboratory and wild batches in the nulliparous category (Figure 5). Fisher's test therefore confirms the result obtained from the ANOVA test.

**3.2. Microbiological Diversity of the Microbiota.** The analysis of Table 5 reveals the presence of the different components of the bacterial microbiota in the abdomen of the female *An. gambiae* from the different batches (wild and laboratory) and in relation to the category (parous, nulliparous, gorged, and nongorged). It can be seen that the results are similar

independently to the pools of mosquito. However, it can be observed in a diversity of mosquitoes' microbiota, whatever is the batch and the category of the mosquito. Thus, bacteria belonging to *Enterobacteriaceae*, *Staphylococcus* spp. and other bacterial genera are found in the abdomen of the analyzed female. However, the nulliparous kdr do not have bacteria belonging to the genus *Enterobacteriaceae*, while sensitive parous Kisumu do not have bacteria belonging to the genus *Staphylococcus*.

**3.3. Confirmation of the Presence of *Spiroplasma* and *Wolbachia*.** The anopheline DNA extracted from batches of female *An. gambiae* mosquitoes (laboratory and wild) was used to test for the presence or absence of *Wolbachia* and *Spiroplasma*. None of the pools displays the presence of *Spiroplasma* spp. As for the search for the *Wolbachia* gene, it was detected among a sample of laboratory *An. gambiae* (sensitive gorged Kisumu) (Table 6).

## 4. Discussion

Bacteria belonging to the *Enterobacteriaceae* and *Staphylococcus* group were identified from *An. gambiae* samples collected. The Gram staining of the different colonies revealed the presence of Gram-positive bacilli, Gram-negative bacilli, and Gram-positive cocci. The presence of Gram-negative bacilli and Gram-positive cocci then confirms the results of the inoculation. In addition, the presence of Gram-positive bacilli suggests the presence of other bacterial genera not identified in this study. There was similarity between the bacterial community of the laboratory mosquitoes (Kdr and sensitive Kisumu) compared to the wild considering the category (parous, nulliparous, gorged, and non-gorged). Regarding the bacterial genus *Staphylococcus* identified during our research, these results are contrary to that of a study carried out in India in 2009 on the

TABLE 3: Results of the analysis of the variance test of the different batches of *Anopheles gambiae* mosquito in the gorged category.

Source of variations	Sum of squares	Degree of freedom	Average of squares	F	Probability (%)	Critical value for F
Between pools	$1.31 \times 10^{12}$	2	$6.54 \times 10^{11}$	1.185	36.83	5.14
Inside the pools	$3.31 \times 10^{12}$	6	$5.52 \times 10^{11}$			
Total	$4.62 \times 10^{12}$	8				

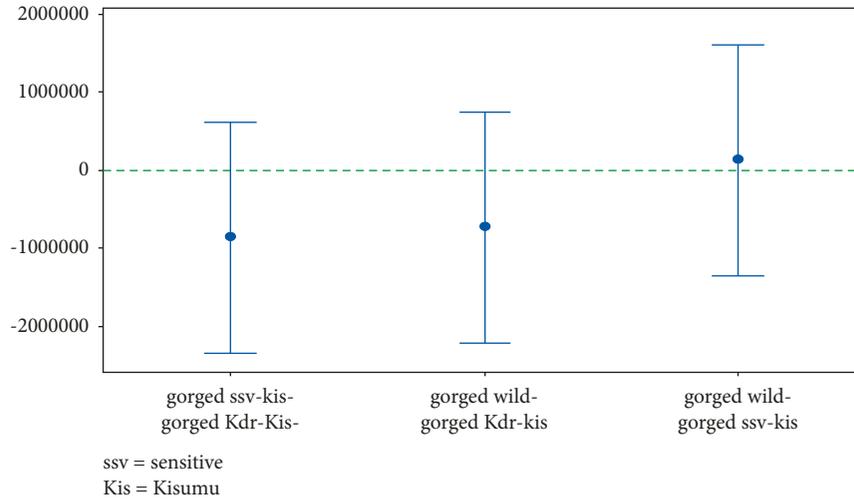


FIGURE 4: Graph of Fisher's test results of gorged mosquitoes.

TABLE 4: Results of the analysis of variance test for different *Anopheles gambiae* according to the category of nulliparous.

Source of variations	Sum of squares	Degree of freedom	Average of squares	F	Probability (%)	Critical value for F
Between pools	$4 \times 10^{14}$	2	$2 \times 10^{14}$	92.04	0.003	5.14
Inside the pools	$1.3 \times 10^{13}$	6	$2.17 \times 10^{12}$			
Total	$4.13 \times 10^{14}$	8				

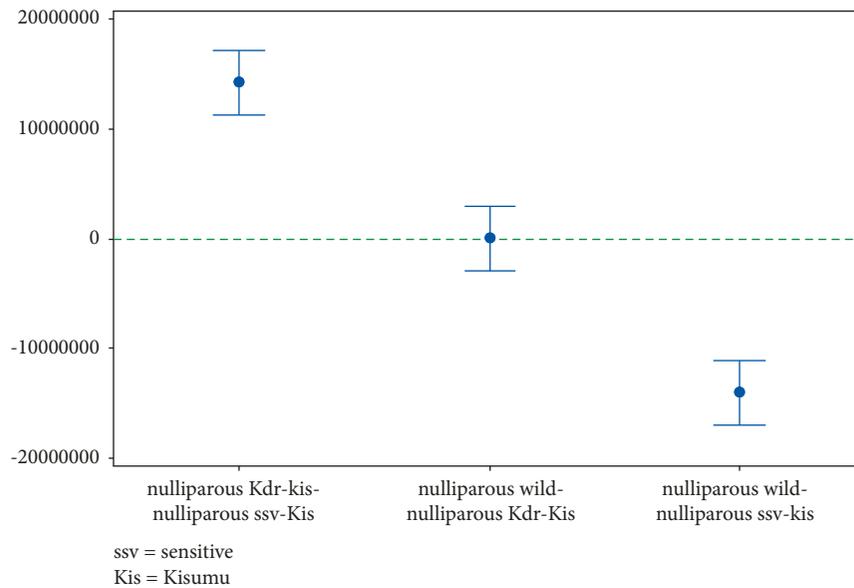


FIGURE 5: Graph of Fisher's test results for nulliparous mosquitoes.

TABLE 5: Microbiological diversity of the microbiota according to batches of mosquitoes.

Pools	Categories	<i>Enterobacteria</i>	<i>Staphylococcus</i>	Other genera	Gram
Kdr	Nongorged	+	+	+	B-, B+, C+
	Parous	+	+	+	B-, B+, C+
	Gorged	+	+	+	B-, B+, C+
	Nulliparous	-	+	+	B-, B+, C+
Kiss	Nongorged	+	+	+	B-, B+, C+
	Parous	+	-	+	B-, B+, C+
	Gorged	+	+	+	B-, B+, C+
	Nulliparous	+	+	+	B-, B+, C+
Wild	Nongorged	+	+	+	B-, B+, C+
	Parous	+	+	+	B-, B+, C+
	Gorged	+	+	+	B-, B+, C+
	Nulliparous	+	+	+	B-, B+, C+

+ = presence; - = absence; B+ = Gram-positive bacillus; B- = Gram-negative bacillus; C+ = Gram-positive cocci; Kdr = Kdr resistant; kiss = sensitive kisumu.

TABLE 6: Search for *Wolbachia* ssp and *Spiroplasma* ssp genes.

Pools	Categories	<i>Wolbachia</i> gene	<i>Spiroplasma</i> gene
Kdr	Parous	-	-
	Nulliparous	-	-
	Gorged	-	-
	Nongorged	-	-
Kisumu	Parous	-	-
	Nulliparous	-	-
	Gorged	-	-
Wild	Nongorged	-	-
	Parous	-	-
	Gorged	-	-
	Nongorged	-	-

+ = positive; - = negative.

field and laboratory populations of the malaria vector *Anopheles stephensi* which revealed the presence of bacteria from the genera *Bacillus* and *Staphylococcus* in males [23]. This difference may be related to the sex of the mosquitoes, the species of the mosquito, and the living environment.

Regarding the *Enterobacteriaceae* family found in our study, our results are similar to those of a study conducted in Kenya [24], but their work does not take to account *Staphylococcus*. This difference observed from the present study could be explained by the difference in sample size or by the ecosystem of rearing laboratory mosquitoes or harvesting wild mosquitoes. However, several studies using metagenomics provide more comprehensive information on the composition of the mosquito midgut microbiota [25,26].

The enumeration of the total mesophilic aerobic flora reveals their presence in both laboratory and wild *An. gambiae*. It has been found that regardless of the nature and category of the mosquitoes, the bacterial load is high. A study conducted in the United States to better understand the potential fluctuations in the microbial load and species composition between laboratory-reared mosquitoes of different generations and within the same generation also showed that the bacterial load is high [8]. Similarly, a study carried out in Kenya indicates that, during the life of an adult, a sharp increase in the bacterial load is observed in

particular [24]. Concerning the comparison of the bacterial load, it was observed that there is no significant difference between the bacterial load of the laboratory and wild mosquitoes in the gorged and nulliparous category. In addition, there is a significant difference between the bacterial load of the laboratory and wild mosquitoes among the nulliparous and nongorged). The gorged and parous laboratory mosquitoes (Kdr and sensitive Kisumu) and the wild ones have in common the taking of blood meals. Vertebrate blood generally contains very few or no germs [27]. Several studies show that the consumption of a blood meal persistently or transiently modifies the composition of the intestinal microbiota through alterations in redox status or metabolism [28,29]. Does the blood test also change the bacterial load? or is it a coincidence? Environmental factors [30], and diet [24], must be considered having an impact on the load and composition of mosquito bacteria.

The research of bacterial genera influencing the *An. gambiae* vectorial capacity revealed the presence of *Wolbachia* in sensitive gorged Kisumu which are mosquitoes reared in the laboratory. Indeed, several studies have revealed the presence of *Wolbachia* in the population of wild mosquitoes, major vectors of malaria transmission, *An. gambiae*'s head and thorax in Mali [31] and ovaries in Burkina Faso [8], and *An. gambiae* and *An. coluzzii* in Gabon [32]. These results are contrary to those provided by this study. This difference can be explained by the limited size of the samples processed or by the fact that the extraction was done only from the abdomen of the mosquitoes in our case. This difference can also be explained by the environment or the season of collecting samples from the wild mosquitoes. In addition, the fragments obtained in the present study are around 500 bp, which suggests a species of *Wolbachia* different from the others obtained in some works. Interestingly, this study shows that, in addition to wild *An. gambiae*, the laboratory ones also harbor *Wolbachia*. The presence of *Wolbachia* has been identified in sensitive gorged *An. gambiae* Kisumu line. So, this presence could be by blood supply or a natural presence within the microbiota. Until now, few studies mention the presence of *Wolbachia* in the blood of vertebrates; however, a study carried out in France shows that the presence of *Wolbachia pipientis* in ticks,

already reported in various publications, was in fact due to the cryptic presence of the endo-parasitoid hymenoptera *Ixodiphagus hookeri* [33]. This association has remained unsuspected until now because parasitoids cannot be detected until the tick nymphs become gorged with blood [33]. In addition, natural infections by *Wolbachia* have been detected in several species of *Anopheles*. For example, in *An. gambiae* in Mali [31] and in *An. gambiae* and *An. coluzzii* in Gabon [32]. However, *Wolbachia* being transmitted vertically by the mother has acquired the particularity of modifying the reproduction of its host. Thus, *Wolbachia* induces cytoplasmic incompatibility [10]. For example, the introduction of *Wolbachia* to *Aedes aegypti*, not only leads to a decrease in infection with pathogens transmissible to humans but can also induce cytoplasmic incompatibility [34]. Some experiments have shown that infection of mosquitoes with *Wolbachia* is not a threat to humans. This bacterium is too large to pass through the salivary duct of mosquitoes into human blood. This makes this bacterium a good candidate for vector control of malaria.

As for the bacterial genera *Spiroplasma*, the research by the technique of PCR was negative for *An. gambiae* for all our samples. However, an investigation of midgut bacteria in *An. gambiae* and *Anopheles funestus* in western Kenya detected *Spiroplasma* in *An. funestus* [14]. In addition, *Anopheles* mosquito microbiome surveys reported *Spiroplasma* from pathogenic clades [14–34].

## 5. Conclusion

It emerges from this study that the major vector of malaria transmission in Benin *An. gambiae* female has a fairly diverse microbiota. It harbors, at high loads, in its midgut, bacteria such as staphylococci, *Enterobacteriaceae*, and other bacterial genera regardless of the nature and its condition. In addition to this, it has been found that, besides the wild mosquitoes, laboratory ones too can be naturally infected with the strains of *Wolbachia* which are known to negatively impact the reproductive organs of their host or even induce cytoplasmic incompatibility. It will thus be useful to continue this study to investigate the effect of symbionts on the life of mosquitoes.

## Data Availability

The data used to support the findings of this work are available from the corresponding author upon request.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

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