



Bacteria Profile of Mosquito Breeding Sites in Some Selected Communities in Plateau State, Central Nigeria

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ABSTRACT

Mosquitoes use different types of water bodies for oviposition. The growth and development of larvae depend on abiotic factors and the larvae's interaction with other organisms in these breeding sites. Amid resistance to available control measures, mosquito-microbe interaction can be exploited for the development of alternative vector control measures. This study focused on the profiling of bacteria composition in mosquito breeding sites within three specific communities in Bokokos Local Government Area of Plateau State, Nigeria. A total of thirty water samples were systematically collected from these communities to analyze the bacterial composition. The aim of this research is to better understand the interaction between mosquitoes and bacteria in their breeding sites, with the ultimate goal of developing alternative vector control measures. This is particularly important given the resistance observed in current control measures. The findings from this study could potentially contribute to the development of more effective strategies for mosquito-borne disease control. Microbial analysis was conducted using culture, microscopy and biochemical test. The result shows that *Klebsiella* spp. and *Providencia* spp. were the most prevalent bacteria isolates found from all the three communities surveyed. *Serratia odorifera* was isolated in Ndar, *Micrococcus* spp., and *Shigella* spp., were isolated in Butura-gida and *Panteoa agglomerans* was isolated only in Kunnet. Other species isolated from the communities include *Bacillus* spp., *Staphylococcus* spp., *Escherichia coli* and *Enterobacter* spp. Bacteria counts in relation to species isolated differed significantly ($\chi^2 = 4897372$, $df = 9$, $P < 0.001$). The differences in bacteria counts for each bacteria isolate in relation to the three selected communities significantly varied ($P < 0.001$). A significant difference ($\chi^2 = 1640.2$, $df = 2$, $P < 0.001$) was observed in total bacteria colony count across locations. In conclusion, investigating the interaction between microbes and mosquitoes could lead to the development of new control methods for mosquito vectors. Most importantly, communities should be educated about the importance of environmental management such as clearing all potential mosquito breeding sites.

Keywords: Bacteria, microbes, mosquito breeding sites, Bokokos LGA, Plateau State, Nigeria

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INTRODUCTION

Various abiotic and biotic factors play a crucial role in influencing the survival of mosquito larvae within their breeding habitats. Factors such as vegetation, temperature, turbidity, pH levels, and concentrations of various compounds including ammonia, salinity, nitrite, nitrate, sulfate, phosphate, chloride, calcium, and water hardness have been shown to impact larval density (Nikookar et al., 2017).

Furthermore, a diverse range of biotic factors including bacteria, fungi, protists, entomopathogenic nematodes, and filamentous fungi contribute to the complexity of breeding sites (Da et al., 2009; Benelli et al., 2016). These biotic factors can act as competitors, predators, or potential food sources for mosquito larvae, thereby significantly influencing their development (Ranasinghe & Amarasinghe, 2020). Notably, numerous studies have demonstrated the essential role of bacteria in mosquito development, as evidenced by high larval mortality in water treated with antibiotics (Touré et al., 2000; Coon et al., 2016). However, conflicting findings have also been reported, with some studies indicating higher survival rates in larvae treated with antibiotics (Santos et al., 2022; Garrigós et al., 2024). This underscores the intricate interplay of various abiotic and biotic factors in shaping the survival and development of mosquito larvae in their respective habitats.

Furthermore, bacteria have been shown to either repel (Falqueto et al., 2021) or attract (Sumba et al., 2004; Ponnusamy et al., 2008; Mosquera et al., 2023;) gravid mosquitoes to potential breeding sites, suggesting they direct ovipositing females. Since the mosquito larvae cannot move from one habitat to another, oviposition site preference of females is considered necessary to ensure the survival and optimum success of the offspring (Girard et al., 2021).

In a study by Sumba et al. (2004), bacteria were found to produce volatiles, specific bacteria-associated carboxylic acids and methyl esters that served as potent oviposition stimulants for gravid *Aedes aegypti*. As repellents, oviposition was significantly reduced when the bacterial colonies of *Stenotrophomonas maltophilia* was present in breeding habitats and no reaction was observed in the presence of bacterial species, *Pseudomonas putida* or *Pseudomonas alcaligenes* (Huang et al., 2006). In another study, a negative correlation was found between larval density and faecal coliform load (Hessou-Djossou et al., 2022). Larvicidal properties of bacteria were investigated in a study where 254 Bacterial strains from different niches were tested for their larvicidal properties on mosquitoes. Eleven were found active against *Aedes aegypti* larvae (Falqueto et al., 2021).

Besides affecting oviposition and development of

larvae, bacteria in the breeding sites also impact the microbiome of adult mosquitoes as bacteria species identified in breeding sites were also identified in larvae and adults (Birnberg et al., 2021). These bacteria that are found in all life stages could confer some advantages to the mosquito.

The interactions between Microbes and mosquitoes are being explored for the development of novel control strategies for mosquito-borne diseases (Ricci et al., 2012; Cansado-Utrilla et al., 2021), an approach recently termed 'symbiotic control' (Ricci et al., 2012). This study hopes to enhance our understanding of larval ecology, which can be harnessed for the development of novel vector control methods targeting mosquito-microbe interactions. This study identified bacteria species found in breeding sites in three selected communities in Bokokos Local Government Area (LGA) of Plateau State, Nigeria, which could be potential biocontrol agents for vectors of mosquito-borne diseases.

MATERIALS AND METHODS

Study area

This study was carried out in three communities in Bokokos LGA, Plateau State. The three communities are Ndar, kunnet and Butura-gida. The communities are dominated by the kulere tribe with a population of over 7000 people. The Plateau state University is situated in Butura-gida with Ndar community to the left and Kunnet to the right. The inhabitants of the community are mostly farmers who cultivate potatoes, maize and vegetables such as tomatoes and cabbage. Mining activities also takes place in the communities.

Habitat characterization

All mosquitoes breeding sites were observed and described in terms of physical condition of the stagnant water and exposure to sunlight (Barros et al., 2011; Afrane et al., 2012; Lapang et al., 2019).

Sample collection and processing

Sterile vial bottles were used to collect water from ground puddles in the three communities. Conditions of the water body, such as exposure to sunlight and vegetation were considered before sample collection. A total of thirty (30) water samples were collected randomly, 10 from each community. The samples were labelled and transported to the National Veterinary Research Institute, Vom. Serial dilutions were performed, and 0.1mL of each sample was taken from a sixfold dilution. All the samples were cultured on Nutrient agar (Oxoid Ltd, Hampshire,

UK) using the spreading method of inoculation and then incubated at 37°C for 24 hours. Identification of the bacteria was done after 24 hours based on their morphological characteristic such as shape and colony appearance. The colonies were sub-cultured on Blood agar and MacConkey agar (Oxoid Ltd, Hampshire, UK) for purification after which biochemical test was done.

Statistical analysis

Data obtained was analyzed using R Console software (Version 4.0.2). Pearson's Chi-square test was used to compare the bacteria counts in relation to the bacteria species, across communities for each isolate observed. Also, total bacteria colony count in relation to locations was compared using Pearson's Chi-square test. The level of significance was set at $P < 0.05$.

RESULTS

Various bacteria isolates found in the breeding sites within the communities

Table 1 shows the various bacterial isolates found in the breeding site within the three communities. A total of nine different bacteria species were isolated. In Ndar five organisms were isolated, *Escherichia coli*, *Klebsiella aerogenes*, *Bacillus* spp., *Staphylococcus* spp., and *Serratia odorifera*. Kunnet had seven isolates, *Escherichia coli*, *Klebsiella aerogenes*, *Staphylococcus aureus*, *Serratia odorifera*, *Providencia rustigianii*, *Bacillus* spp., and *Panteoa agglomerans*. While in Butura-gida, *Bacillus* spp., *Enterobacter* spp. *Providencia* spp., *Shigella* spp., *Staphylococcus* spp., and *Micrococcus* spp. were isolated.

Gram reaction and shape

Table 2 provides an overview on Gram-reaction of the isolates after staining. The study revealed Four (4) species of gram-positive bacteria. All were rod shaped except *Staphylococcus* spp. which was cocci. Four organisms were gram-negative, and the others were gram-positive.

Table 1: Various bacteria isolates found in the different communities.

Location	Bacteria Isolate
Ndar	<i>Escherichia coli</i> , <i>Klebsiella aerogenes</i> , <i>Bacillus</i> spp., <i>Staphylococcus</i> spp., <i>Serratia odorifera</i>
Kunnet	<i>Escherichia coli</i> , <i>klebsiella aerogenes</i> , <i>Staphylococcus aureus</i> , <i>Serratia odorifera</i> , <i>Providencia rustigianii</i> , <i>Bacillus</i> spp., <i>Panteoa agglomerans</i>
Butura Gida	<i>Bacillus</i> spp., <i>Enterobacter</i> spp., <i>Providencia</i> spp., <i>Shigella</i> spp., <i>Staphylococcus</i> spp., <i>Micrococcus</i> spp.

Table 2: Gram reactions and shape of bacteria isolates from mosquitoes breeding sites in Bokkos LGA, Plateau State.

Location	Gram Reaction	Shape	Isolate
Ndar	-	Rod	<i>Escherichia coli</i>
	-	Rod	<i>Klebsiella aerogenes</i>
	+	Rod	<i>Bacillus</i> spp.
	+	Cocci	<i>Staphylococcus</i> spp.
	-	Rod	<i>Serratia odorifera</i>
Kunnet	-	Rod	<i>Escherichia coli</i>
	-	Rod	<i>Klebsiella</i> spp.
	+	Rod	<i>Bacillus</i> spp.
	+	Cocci	<i>Staphylococcus</i> spp.
	-	Rod	<i>Serratia</i> spp.
	+	Rod	<i>Providencia</i> spp
	+	Rod	<i>Panteoa agglomerans</i>
Butura-gida	+	Rod	<i>Bacillus</i> spp.
	+	Cocci	<i>Staphylococcus</i> spp.
	+	Rod	<i>Providencia</i> spp.
	-	Rod	<i>Enterobacter</i> spp.
	-	Rod	<i>Shigella</i> spp.
	+	Cocci	<i>Micrococcus</i> spp.

Table 3: Morphological characteristic (Shape, Elevation and Margin) of bacteria colony

Habitat	Shape	Elevation	Margin	Isolate
Ndar	Circular	biconvex	Entire	<i>Escherichia coli</i>
	Circular	Convex	Entire	<i>Klebsiella</i> spp.
	Circular	Flat	Irregular	<i>Bacillus</i> spp.
	Circular	Convex	Entire	<i>Staphylococcus</i> spp.
	Circular	Convex	Entire	<i>Serratia odorifera</i>
Kunnet	Circular	Low-convex	Entire	<i>Escherichia coli</i>
	Circular	Convex	Entire	<i>Klebsiella</i> spp.
	Circular	Convex	Irregular	<i>Bacillus</i> spp.
	Circular	Convex	Entire	<i>Staphylococcus</i> spp.
	Circular	Convex	Entire	<i>Serratia</i> spp.
	Circular	Convex	Regular	<i>Providencia</i> spp.
	Circular	Convex	Entire	<i>Panteoa agglomerans</i>
Butura-gida	Circular	Convex	Irregular	<i>Bacillus</i> spp.
	Circular	Convex	Entire	<i>Staphylococcus</i> spp.
	Circular	Convex	Regular	<i>Providencia</i> spp.
	Circular	Convex	Entire	<i>Enterobacter</i> spp.
	Circular	Convex	Entire	<i>Shigella</i> spp.
	Circular	Convex	Entire	<i>Micrococcus</i> spp.

Table 4: Morphology of the bacteria isolate on blood agar

Habitat	Size of colonies	Color	Isolate
Ndar	Small	Greyish	<i>Escherichia coli</i>
	Large	Mucoid-greyish	<i>Klebsiella pneumonia</i>
	Small	Greyish	<i>Bacillus</i> spp.
	Small	Creamy	<i>Staphylococcus</i> spp.,
	Small	Greyish	<i>Serratia odorifera</i>
Kunnet	Small	Greyish	<i>Escherichia coli</i>
	Large	Mucoid-greyish	<i>Klebsiella aerogene</i>
	Small	Greyish	<i>Bacillus</i> spp.
	Small	Creamy	<i>Staphylococcus aureus</i>
	Small	Greyish	<i>Serratia odorifera</i>
	Small	Brownish	<i>Providencia rustiginaii</i>
	Small	Greyish	<i>Panteoa agglomerans</i>
Butura-gida	Small	Greyish	<i>Bacillus</i> spp.
	Small	Creamy	<i>Staphylococcus</i> spp.
	Small	Greyish	<i>Providencia</i> spp.
	Small	Greyish	<i>Enterobacter</i> spp.
	Small	Creamy	<i>Shigella</i> spp.
	Small cluster	Yellowish	<i>Micrococcus</i> spp.

Morphological characteristics of bacteria colony

Table 3 shows the morphological characteristics of the organism on nutrient agar giving their shape, elevation and margins. All organisms were circular in shape with only *Bacillus* spp. having an irregular margin.

Morphology of the bacteria isolate on blood agar

Table 4 shows the morphological characteristics of organisms on blood agar. It shows the size and colour of isolates. All colonies were small except *Klebsiella pneumonia* isolate from Ndar and Kunnet community.

Counts of various bacteria isolates from the three selected communities in Bokkos LGA, Plateau State

Table 5 shows the counts of the ten different isolates

pooled from the 3 selected communities in Bokkos LGA of Plateau State expressed in Cfu/ml. In overall, the bacterial isolate with the most dominant total mean bacteria count was *Klebsiella* spp. (2.7×10^6) followed by *Providencia* spp. (2.65×10^6), *Enterobacter* spp. and *Panteoa* spp. (2.0×10^6), *Staphylococcus* spp. (1.76×10^6), *Bacillus* spp. (1.42×10^6), *Escherichia coli* (7.0×10^5), *Shigella* spp. (6.0×10^5), *Serratia* spp. (5.2×10^5), and *Micrococcus* spp. (3.0×10^5), respectively, with significant difference ($\chi^2 = 4897372$, $df = 9$, $P < 0.001$). *Staphylococcus* spp. had the highest count in Ndar (8.3×10^5) and Butura-gida (6.3×10^5) communities. In Kunnet, *Serratia* spp had the highest count (4.2×10^5). The differences in bacteria counts for each isolate in relation to the 3 selected communities differed significantly ($P < 0.001$) as shown in (Table 5).

Table 5: Counts of various bacterial isolates from three selected communities in Bokkos LGA, Plateau State.

Bacterial Isolate	Total bacteria count (mean value) (Cfu/ml) across communities			χ^2	df	P-value
	Ndar	Kunnet	Butura-gida			
<i>Escherichia coli</i>	5.0×10^5	2.0×10^5	0	542857	2	0.001*
<i>Klebsiella</i> spp.	6.0×10^5	2.1×10^6	0	2600000	2	0.001*
<i>Bacillus</i> spp.	6.0×10^5	3.2×10^5	5.0×10^5	85070	2	0.001*
<i>Staphylococcus</i> spp.	8.3×10^5	3.0×10^5	6.3×10^5	244205	2	0.001*
<i>Serratia</i> spp.	1.0×10^5	4.2×10^5	0	555385	2	0.001*
<i>Providencia</i> spp.	0	2.3×10^6	3.5×10^5	3477358	2	0.001*
<i>Enterobacter</i> spp.	0	0	2.0×10^5	4000000	2	0.001*
<i>Shigella</i> spp.	0	0	6.0×10^5	1200000	2	0.001*
<i>Micrococcus</i> spp.	0	0	3.0×10^5	600000	2	0.001*
<i>Pantoea</i> spp.	0	2.0×10^6	0	4000000	2	0.001*

*: Significant

Mean Microbial load of Mosquito Breeding Sites in different Communities

Table 6 shows the total bacteria colony count found in relation to the three communities surveyed. The total bacteria colony count in order of locations dominance was Ndar (3.3×10^2) > Butura-gida (2.6×10^3) > Kunnet (1.9×10^3). Thus, a very high significant difference ($\chi^2 = 1640.2$, $df = 2$, $P < 0.001$) was observed in total bacteria colony count across locations.

Table 6: Mean bacterial load of mosquito breeding sites in the three selected communities.

Locations	Number of samples	Total bacteria count (Mean value) (Cfu/ml)
Ndar	10	3.5×10^2
Kunnet	10	1.9×10^3
Butura-gida	10	2.6×10^3

DISCUSSION

Several studies have reported on the interaction between microorganisms and mosquitoes. These microorganisms have been observed to act as stimulants or repellants for mosquito oviposition, as well as serving as food sources and occasional predators of mosquitoes (Ranasinghe & Amarasinghe, 2020). Furthermore, the presence of microorganisms in mosquito breeding sites has been found to impact the development of larvae, ovipositing females, and the bacterial composition of the mosquito gut (Nilsson et al., 2019). Notably, our study identified several species of bacteria, particularly those belonging to the Bacillaceae family, which have been associated with various mosquito species known to transmit public health-important diseases. *Staphylococcus* spp and *Bacillus* spp were found in the three communities with the highest abundance in Ndar. Several *Bacillus* spp. have shown potential as biocontrol agents against mosquito vectors; for instance, *Bacillus thuringiensis israelensis* (Bti) is considered safe and effective against many mosquito species, therefore used for biocontrol purposes

(Katak et al., 2023). Bti has been shown to effectively reduce the population of *Aedes* larvae however, for effective long-term control, incorporating other control measures might be required (Boyce et al., 2013). In a study by Fouda et al. (2001), *Bacillus* spp. and *Staphylococcus* spp. were found in the midgut of adult *Culex pipiens* and are considered to influence fertility.

Shigella spp., *Enterobacter* spp. and *Micrococcus* spp. were isolated from Butura-gida. The distribution of these bacteria could be as the result of human activities within the habitat. In a study where the microbiota of four breeding sites for *Anopheles darlingi* were explored, *Escherichia*, *Shigella*, *Staphylococcus*, and *Pseudomonas* were reported to be the most prevalent suggesting that the most common microorganisms in all breeding sites could be contributing to making the habitat conducive to the mosquito vector in all study sites (Nilsson et al., 2019).

Escherichia coli were found in Ndar and Kunnet. In a study conducted in Thailand and Laos, a higher abundance of *Escherichia coli* in breeding waters was strongly correlated to the presence of *Aedes Aegypti* mosquitoes (Dada et al., 2013). *Serratia* spp. and *Klebsiella* spp. were also isolated in our study. A study in Rwanda revealed the presence of *Asaia*, *Wolbachia* and *Serratia* species in abundance. These bacteria are considered potentials in the control of arboviruses (Tokash-Peters et al., 2024). In a study in Imo state, *Klebsella* spp, *Pseudomonas* spp., *Bacillus* spp. and *Acinetobacteria* spp. were also isolated from ground puddles (Udujih Obinna Godwin et al., 2021). *Klebsiella* spp. have been reported to be associated with *Aedes*, *Anopheles* and *Culex* spp mosquitoes (Mosquera et al., 2021; Rocha et al., 2021). *Klebsiella* spp. have also been isolated from water from natural breeding sites and containers (Rocha et al., 2021). *Klebsiella* spp. has also been isolated from the midgut of *An. gambiae* in a study conducted by Jadin et al. (1966), the study shows that *Klebsiella* spp. are pathogenic to *Anopheles gambiae* and *Anopheles stephensi*. The organism has also been shown to stimulate oviposition in *Culex* spp. (Díaz-Nieto

et al., 2016). *Serratia* spp. (Bando et al., 2013) and *Escherichia coli* (Tchioffo et al., 2016) have been shown to inhibit the development of *Plasmodium* within the mosquito midgut. Research into alternative methods for controlling mosquito-borne diseases has become imperative due to the development of insecticide resistance by mosquitoes. Microbes have emerged as potential substitutes for synthetic insecticides, prompting the need to comprehensively understand the interactions between microorganisms, mosquitoes, pathogens, and the environment in order to achieve successful outcomes (Katak et al., 2023). Further investigation into the impact of the microbe-mosquito relationship is crucial for gaining insights into how this interaction influences the phenotype of mosquitoes, as well as their capacity to survive, reproduce, and transmit diseases (Cansado-Utrilla et al., 2021).

Conclusion

The findings of this study highlight the potential for targeting mosquito breeding sites as a strategy for controlling mosquito-borne diseases. It has been observed that the presence of certain microbes in these breeding sites significantly affects the survival and normal functioning of mosquitoes. Therefore, further research into the relationship between these microbes and mosquitoes in breeding sites is essential for enhancing our understanding of larval ecology and developing alternative methods for vector control. Community engagement plays a critical role in the efforts to combat malaria and other mosquito-borne diseases. It is important to organize sensitization programs to raise awareness about the negative impact of mosquito breeding sites within and around communities. Disrupting the presence of certain microbes that have a positive influence on mosquito survival could have adverse effects on vector populations. On the other hand, microorganisms that are detrimental to mosquitoes could be explored as alternatives to synthetic insecticides.

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