



Bacteria and fungi profile of mosquito oviposition sites in Eziobodo, Owerri West L.G.A., Imo State

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Open Access Research Journal of Science and Technology, 2021, 01(01), 024–028

Publication history: Received on 31 January 2021; revised on 01 March 2021; accepted on 04 March 2021

Article DOI: <https://doi.org/10.53022/oarjst.2021.1.1.0021>

Abstract

Microorganisms in breeding sites may act as important factors for attracting or repelling mosquitoes. This study investigated the presence and counts of bacteria and fungi isolates from mosquito breeding sites in Eziobodo Community of Owerri West Local Government Area of Imo State, Nigeria. The work was carried out by collecting 5 water samples each from randomly selected breeding site types in the study area. Microbial analysis of the water samples were done using culture, microscopy and biochemical techniques. The breeding sites identified in the study include Tireopenings, ground puddles, Generator house area, and open containers. The bacteria isolated were *Bacillus sp*, *Acinetobacter sp*, *Staphylococcus sp*, *Pseudomonas sp*, *Escherichia coli*, and *Klebsiella sp* while the fungi isolated were *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus* and *Candida sp*. *Acinetobacter sp* showed the highest counts in ground puddles and open containers, *Staphylococcus sp* showed highest counts in Tire openings whereas, in Generator houses only *staphylococcus sp* and *Escherichia coli* were the only bacteria isolated. Awareness on the importance of environmental management among residents in the control of mosquitoes as well as disruption of microbial activities in breeding sites could be included in current measures to control mosquito vector borne diseases in the study area.

Key Words: Mosquitoes; Bacteria; Fungi; Breeding Sites; Eziobodo

1. Introduction

The location and selection of an oviposition site is an essential life-cycle behavior for all mosquito species and involves visual, olfactory, and tactile cues [1]. The vectorial capacity of mosquitoes is due, in a large part, to their rapid and high levels of reproduction. It appears that a female mosquito will not oviposit into every available water source, or even into every suitable one, but identifying which sites a mosquito will deposit her eggs into has proven difficult as habitats where larvae are found seem to have few distinguishing features that mark them out easily from un-colonised sites [2].

There are many cues that regulate oviposition in mosquitoes. Internal factors include the circadian rhythm, which determines when in the 24hr day a mosquito will oviposit [3;4], hormones, which regulate the development of eggs and other process [5], and the taking of a blood meal, which can alter gene expression [6]. The physiological state of the mosquito is, therefore, important in determining if and when oviposition occurs, but where it occurs is more dependent on external factors.

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The oviposition of many mosquitoes appears to be mediated, at least in part, by semiochemicals. Semiochemical is a broad term relating to any chemical (or mixtures of chemicals) which mediate interactions between organisms. Semiochemicals are largely divided into two groups; pheromones, which are intraspecific, and allelochemicals, which are interspecific and therefore affect species other than their originator [7]. Bacteria and volatiles from bacteria have been shown to affect mosquito behaviour in a number of studies. For example, human skin microflora is believed to be one of the underlying causes of the observed differences in human attractiveness to mosquitoes [8;9]. Apart from host-seeking responses, several bacterial-produced volatiles may function as oviposition attractants/stimulants for different mosquito species.

Bacteria are important food sources for mosquito larvae. It is not possible to rear *Aedes aegypti* (L) larvae in sterile media [10]. In agreement, *An. gambiae* larval development was hampered in rearing water containing Gentamycin and Penicillin-Streptomycin where a majority of the larvae died before pupation [11]. In addition, *An. gambiae* and *Anopheles quadrimaculatus* (Theobald) larvae, when reared in the presence of Gentamycin, were significantly smaller than larvae reared without Gentamycin [12]. Taken together, these studies show that bacteria are important, if not essential, for the development of the larvae of several species of mosquitoes.

The importance of this study is to increase our understanding of mosquito oviposition, in order to better understand how the behaviour of the mosquito can be exploited to reduce mosquito populations. It aims to explore the bacteria profile of oviposition site with the hope that quantification of behavioural responses to oviposition site cues will allow the mosquito to be controlled before it can become an irritation as a biting nuisance, *let alone* a disease vector.

2. Material and methods

2.1. Study area

The study was carried out in Eziobodo, Owerri-West, Imo State. Eziobodo is an ancient kingdom located in today's Owerri-West Local Government, Owerri - Imo State Nigeria. Eziobodo is also known as Alueze or Alu-eze as this was the ancient name of the community before the colonial arrival of the former British Empire and Warrant Chiefs that followed much later around the 1930's. It is an igbo-speaking community with a population of over 15,000 locals. The town hosts the Federal University of Technology Owerri - FUTO. Eziobodo accounts for over 70% of the landmass of Federal University of Technology, Owerri. The town is also known for agricultural produce such as cassava, pumpkin leaf (Ugu), cocoyam, maize, and palm oil.

2.2. Study Population

The target population of this study consist of inhabitants of some 71 households in Eziobodo Community, Owerri-West, Imo State.

2.3. Sample collection and analysis

Sample collection was done using a sterile bottle to collect water samples from mosquitobreeding sites in Eziobodo Community. Twenty water samples (five each of the various breeding sites identified) were collected randomly from various compounds. It was necessary to transport the sample using icebox to keep them cool enough to retard changes in bacteria density. All samples were cultured immediately after arrival to the laboratory. Identification of Bacteria was based on morphological characteristics observed for each bacteria colony after 24hrs of growth including colony appearances, shape. Biochemical characterization were done using some key tests for identification [13]

The fungi colonies were sub-cultured on Sabouraud dextrose agar (SDA). The isolates were identified based on their morphological and microscopic features.

3. Results

3.1. Various bacterial isolates found in the breeding sites

Table 1 shows the various bacterial isolates found in the breeding sites around households in Eziobodo Community. In these various breeding sites such as Tireopenings, *Bacillus sp* and *Staphylococcus sp* were isolated. In open stagnant water, *Klebsiella*, *Pseudomonas*, *Bacillus sp* and *Acinetohacter sp* were isolated. In Generator house area, *Escherichia coli* and *Staphylococcus sp* were isolated. While in open containers, *Klebsiella sp*, *Pseudomonas sp*, *Bacillus sp*, *Acinetohacter sp* and *Staphylococcus sp* were also isolated.

Table 1 Various bacterial isolates found in the breeding sites

Breeding site	Bacterial isolates
Tire opening	<i>Bacillus, Staphylococcus</i>
Ground Puddle	<i>Klebsiella, Pseudomonas, Bacillus, Acinetohacter</i>
Generator house area	<i>Escherichia Coli, Staphylococcus</i>
Open Containers	<i>Klebsiella, Pseudomonas, Bacillus, Acinetohacter, Staphylococcus</i>

3.2. Various Fungal Isolates Found In the Breeding Sites

Table 2 shows the various fungi isolates found in the breeding sites. In Tireopening were *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus* and *Candida spp* were isolated. In open stagnant water, *Aspergillus niger* was isolated. In Generator house area, no fungal organism was isolated while in open containers, *Candida spp* was isolated.

Table 2 Various fungal isolates found in the breeding sites

Breeding site	Fungi isolates
Tire opening	<i>Aspergillus niger, aspergillus flavus, Aspergillus fumigatus, Candida spp.</i>
Ground puddles	<i>Aspergillus niger</i>
Generator house area	<i>Nil</i>
Container site	<i>Candida spp.</i>

3.3. Counts of Various Isolates Expressed In Cfu/ml

Table 3 shows the counts of various isolates expressed in Cfu/ml. *Acinetobacteria spp* had the highest counts in breeding sites (Ground puddle and open containers) where they were present. *Staphylococcus spp* had the highest counts (1.3×10^2) in Tireopenings whereas *Pseudomoas spp* had the second highest count in open containers. *Klebsiella spp* and *Staphylococcus spp* had the least counts in ground puddle (1.0×10^1) and open containers (1.0×10^1).

Table 3 Counts of various isolates expressed in Cfu/ml

Bacterial isolates	Total Bacteria count (mean value) (Cfu/ml)			
	Ground puddle	Tireopening	Generator house	Open Containers
<i>Klebsella spp.</i>	1.0×10^1	-	-	2.0×10^1
<i>Pseudomonas spp.</i>	2.4×10^2	-	-	4.1×10^2
<i>Bacillus spp.</i>	2.0×10^1	1.0×10^1	-	3.0×10^1
<i>Staphylococcus spp.</i>	-	1.3×10^2	4.5×10^1	1.0×10^1
<i>Acinetobacteria spp.</i>	8.5×10^2	-	-	8.3×10^2
<i>Escherichia coli</i>	-	-	3.5×10^1	-

3.4. Mean Microbial Load of Mosquito Breeding Sites

The table 4 shows the mean microbial load of mosquito breeding sites. In ground puddle, total bacterial count was 1.1×10^2 , while total fungal count was 2.0×10^1 . In Tire opening, total bacterial count was 1.4×10^2 , while total fungal count 6.0×10^1 . In Generator house area, total bacterial count was 8.0×10^1 , while total fungal count was zero (0). In container site total bacterial count was 1.3×10^3 , while total fungal count was zero.

Table 4 Mean microbial load of mosquito breeding site

Breeding sites	No of samples	Total bacterial count (mean value) Cfu/ml	Total fungal count (mean value) Cfu/ml
Ground puddle	5	1.1×10^2	2.0×10^1
Tire opening	5	1.4×10^2	6.0×10^1
Generator house area	5	8.0×10^1	Nil
Container site	5	1.3×10^3	Nil

4. Discussion

Several studies have determined bacteria as larval food, mid gut flora and its metabolites to be an effective oviposition attractant and / or stimulant in mosquitoes using different methods [8; 14; 15; 16]. The findings in this study showed the presence of bacteria and fungi in mosquito breeding sites in the study area.

Acinetobacter sp showed the highest counts in ground puddles and open containers, *Staphylococcus sp* showed highest counts in tire openings whereas, in generator houses only *staphylococcus sp* and *Escherichia coli* were the only bacteria isolated. These distributions maybe as a result of human activity and natural occurrence of bacteria in the environment. The microorganisms isolated from the breeding sites have been shown to be associated with different mosquito species which are vectors of various diseases of public health importance. *Pseudomonas aeruginosa* in a study produced an oviposition attractant/stimulant for *Aedes aegypti* and *Culex pipiens* [17]. In studies on oviposition responses of *Aedes* mosquitoes in different types of water, it was observed that *Aedes aegypti* preferred to oviposit in stagnant water that contained *Acinetobacter anitratus*. In addition, *Anopheles gambiae* and *Anopheles quadrimaculus* (Theobald) larvae when reared in the presence of Gentamycin were significantly smaller than larvae reared without Gentamycin [12; 11] showing evidence that bacteria in breeding sites affect mosquito larvae development. Some studies have investigated the correlation of mainly gram-negative bacteria in the midgut was reported to either completely or partially inhibit parasite development [18; 14]. In contrast, one study reported a correlation between the presence of *Pseudomonas sp* and enhanced development of *Plasmodium* [19].

Taken together, these studies support the theory that volatiles emitted by bacteria are utilized as semiochemicals by mosquitoes in oviposition behaviour. Since various groups of bacteria involved in eliciting the oviposition response aid mosquito species by guiding them to the oviposition site and so ensuring their breeding and continued presence in the community. If therefore, the breeding water bacteria can be controlled, it will surely be a success in controlling the mosquitoes without any environmental hazards. Hence disease control through targeted disruption of microbial activities or proliferation in oviposition sites could prove effective.

5. Conclusion

The present study clearly demonstrates the presence of bacteria associated with mosquito vectors in the study area. It is also suggestive of the presence of breeding habitats in the study area. It is therefore necessary to create awareness on the importance of environmental management for the control of mosquitoes among residents in the study area. In addition, disruption of microbial activities in breeding sites could be included in current measures to control mosquito vector borne diseases.

Compliance with ethical standards

Acknowledgments

We acknowledge the leaders and residents of Eziobodo community for granting consent and access

Disclosure of conflict of interest

We declare there is no conflict of interest

References

- [1] Day, J.F. (2016). Mosquito Oviposition Behavior and Vector Control. *Insects*, 7, 65.
- [2] Majambere, S, Fillinger, U, Sayer, D, Green, C and Lindsay, SW. Spatial distribution of mosquito larvae and the potential for targeted larval control in The Gambia. *American Journal of Tropical Medicine and Hygiene*, 2008; 79:19-27.
- [3] Sumba, LA, Guda, TO, Deng, AL, Hassanli, A, Beier, JC and Knols, BGJ. Mediation of oviposition site selection in the African malaria mosquito *Anopheles gambiae* (Diptera: Culicidae) by semiochemicals of microbial origin. *International Journal of Tropical Insect Science* 2004; 24:260-265.
- [4] Fritz, ML, Huang, J, Walker, ED, Bayoh, MN, Vulule, J and Miller, JR. Ovipositional periodicity of caged *Anopheles gambiae* individuals. *Journal of Circadian Rhythms*, 2008; 6:2.
- [5] Clements, AN. *The Biology of Mosquitoes Volume 1, Development, Nutrition, Reproduction*. Chapman and Hall, London, United Kingdom. 1992
- [6] Rinker, DC, Pitts, RJ, Zhou, X, Suh, E, Rokas, A and Zweibel, LJ. Blood meal-induced changes to antennal transcriptome profiles reveal shifts in odor sensitivities in *Anopheles gambiae*. *Proceedings of the National Academy of Science*, 2013; 110:8260-8265.
- [7] Brown, W L, Eisner, T and Whitaker, W H. Allomones and kairomones: Transspecific chemical messengers. *BioScience*, 2007; 20:21-22.
- [8] Braks, M A H, Scholte, E J, Takken, W and Dekker, T. Microbial growth enhances the attractiveness of human sweat for the malaria mosquito, *Anopheles gambiae sensu stricto* (Diptera: Culicidae). *Chemoecology*, 2000; 10:129-134.
- [9] Takken, W. The sensory physiology of host-seeking behavior in mosquitoes. *Annual Review of Entomology*, 1991; 36:139-158.
- [10] Merritt, RW, Dadd, R H and Walker, ED. Feeding-behavior, natural food, and nutritional relationships of larval mosquitos. *Annual Review of Entomology*, 1992; 37:349-376.
- [11] Touré, AM, Mackey, AJ, Wang, ZX and Beier, JC. Bactericidal effects of sugar-fed antibiotics on resident midgut bacteria of newly emerged anopheline mosquitoes (Diptera: Culicidae). *Journal of Medical Entomology*, 2000; 37:246-249.
- [12] Wotton, RS, Chaloner, DT, Yardley, CA and Merritt, RW. Growth of *Anopheles* mosquito larvae on dietary microbiota in aquatic surface microlayers. *Medical and Veterinary Entomology*. 1997; 11:65-70.
- [13] Cheesbrough, M. *District Laboratory Practice in Tropical Countries 2nd Edition update Part 1*. Cambridge University Press Edinburgh, UK. 2010; Pp 239-244.
- [14] Gonzalez-Ceron, L, Santillan, F, Rodriguez, MH, Mendez, D and Hernandez-Avila, JE. Bacteria in midguts of field-collected *Anopheles albimanus* block *Plasmodium vivax* sporogonic development. *Journal of Medical Entomology*, 2003; 40:371-374.
- [15] Pereira, E DS, Sarguis MID, Fereira-Keppler, R.L, Hamada, N, Alencor, Y. Filamentous fungi associated with mosquito larvae (Diptera: Culicidae) in Municipalities of the Brazilian Amazon. *Neotropical Entomology*. 2009; 38:352-222.
- [16] Chikalo, E and Abate, D. Bacterial Populations of mosquito breeding habitats in relation to maize pollen in Asendabo, South Western Ethiopia. *African Journal of Microbiology Research*. 2010; 55-64.
- [17] Lindh, JM, Kannaste, A, Knols, BGJ, Faye, I and Borg-Karlson, AK. Oviposition Responses of *Anopheles gambiae*s. (Diptera: Culicidae) and Identification of Volatiles from Bacteria-Containing Solutions. *Journal of Medical Entomology*, 2006; 45:1039-1049.
- [18] Pumpuni, CB, Demaio, J, Kent, M, Davis, JR and Beier, JC. Bacterial population dynamics in three anopheline species: the impact on *Plasmodium* sporogonic development. *American Journal of Tropical Medicine and Hygiene*, 1996; 54:214-218.
- [19] Jadin, J, Vincker, IH, Dunjic, A, Delville, JP, Wery, M, Bafort, J and Scheepers- Biva, M. Role of *Pseudomonas* in the sporogenesis of the hematozoon of malaria in the mosquito. *Bulletin of Sociology and Pathology* 1966; 59:514-525