



EXTENSIVE RESISTANCE OF ANOPHELES SP. MOSQUITOES IN NON-MALARIA CONTROL FOCUS AREAS

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Abstract

Resistance to insecticides is a global phenomenon that is felt by all stakeholders in developed and developing countries. Insecticide resistance is the main barrier to long-term malaria control and can be exacerbated by the uncontrolled use of agricultural and household pesticides. This study used a cross-sectional method to test *Anopheles* sp mosquitoes using the CDC Bottle Assay insecticide resistance test with bendiocarb (Ficam 80 WP) 12.5 µg and lambda-cyhalothrin (Icon 100 CS) 12.5 µg. Implementation of the resistance status test on the death rate at 30 minutes in the laboratory. Resistance status of *Anopheles*

sp. mosquitoes to test insecticides in the category of non-controlling areas in Lebakwangi Village, Banjarnegara Regency resulted in an unexpected condition of resistance to bendiocarb (Ficam 80 WP) 12.5 µg and lambda-cyhalothrin (Icon 100 CS) 12.5 µg. Analysis of differences in resistance status of *Anopheles* sp. mosquitoes in the category of controlled areas and the category of non-controlled areas in Banjarnegara District showed no significant difference. This study contributes to the description and warning that the existing types of insecticides have expanded to the resistance conditions of malaria vectors. This data may assist future Malaria Control Programs for decision making regarding vector control planning and resistance management. A

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Introduction

The World Health Organization (WHO) has provided estimates indicating that in 2019, there were approximately 229 million cases of malaria and 409,000 malaria-related deaths worldwide (WHO, 2019). To combat malaria vectors on a global scale, WHO recommends implementing strategies such as the increased utilization of insecticide-treated nets (LLINs) and the application of residual pesticide spraying within rooms (IRS) (Glunt et al., 2015; Kitau et al., 2014; Permenkes, 2007).

Malaria control policies encompass various measures, including the provision of intermittent preventive treatment (IPT) to pregnant women and the distribution of sufficient LLINs to children aged 0-5 years, in accordance with WHO guidelines (World Health Organization, 2020). The implementation of insecticide-based malaria vector control programs at a global level has resulted in various consequences for *Anopheles* mosquito populations, such as physiological adaptations and behavioral changes (Ahmad et al., 2016; Ranson & Lissenden, 2016). Continuous selective pressure on mosquito populations prompts a new process of adaptation to environmental conditions. Notably, in regions where pesticides are extensively employed for malaria vector control, there has been an observed increase in tolerance to organophosphate and carbamate insecticides (Dabiré et al., 2014).

This resistance to insecticides in malaria vectors primarily arises from an augmented activity of detoxifying enzymes that enhance metabolic processes. Mosquitoes possess a remarkable ability to adapt to environmental conditions, including the presence of insecticides, which renders them susceptible to various types of insecticides. Consequently, the use of pesticides in malaria vector control has led to the development of tolerance to these chemicals. Furthermore, specific classes of pesticides employed in malaria vector control have proven ineffective and necessitate replacement with alternative insecticides.

Monitoring efforts to assess resistance levels are primarily focused on regions where control activities are conducted, while limited attention is given to areas beyond



these focal points. Understanding the mechanisms underlying resistance is crucial for the success of vector control strategies. Therefore, it is essential to describe and examine the distribution patterns of resistance in areas lacking specific malaria vector control efforts and compare them to regions where such control measures have been implemented.

Methods

This study employed a cross-sectional research design to investigate adult *Anopheles* sp mosquitoes in malaria-endemic regions of Banjarnegara Regency. The research targeted two distinct sample groups: *Anopheles* sp mosquitoes collected from malaria-endemic areas where spraying/indoor residual spraying (IRS) had been extensively implemented, and *Anopheles* sp mosquitoes from areas where no spraying/IRS activities had been carried out. The study locations comprised Gunungjati Village, representing areas with historical IRS applications, and Lebakwangi Village, representing areas without such interventions. To assess the resistance levels of the sampled mosquitoes, the researchers employed the CDC Bottle Assay method. This technique involved placing 10-25 mosquitoes in each test bottle for both control and treatment groups. For the resistance tests, the researchers used two insecticides: bendiocarb (Ficam 80 WP) with a concentration of 12.5 µg/bottle and lambda cyhalothrin (Icon 100 CS) with a concentration of 12.5 µg/bottle. The breeding sites of mosquitoes were taken into account, including their proximity to human habitation and agricultural sites within the research area, as factors that may influence the resistance patterns.

Results and Discussion

The investigation into the resistance status of *Anopheles* sp mosquitoes to bendiocarb (12.5 µg) and lambda-cyhalothrin (12.5 µg) in Gunungjati and Lebakwangi Villages, Banjarnegara District revealed no significant difference in resistance status



between areas with and without indoor residual spraying (IRS) interventions. In the past decade, malaria vector control in Banjarnegara District has relied on the implementation of IRS using various insecticide derivatives from the pyrethroid and carbamate groups, with a rotation scheme of every 2-3 years following application. The emergence of resistance in *Anopheles* sp mosquitoes collected from Banjarnegara Regency can be attributed to a combination of factors, ultimately compromising the efficacy of insecticide use. Both biological and operational factors contribute to the development of resistance in mosquitoes. Pesticide-based vector control activities eliminate susceptible individuals within the vector population, allowing resistant individuals to survive and propagate. Furthermore, the application of insecticides in residue form accelerates the rate of resistance due to increased contact between the active ingredients and *Anopheles* sp mosquitoes compared to other application methods (Ahmad et al., 2016). Gunungjati and Lebakwangi Villages, situated in Banjarnegara District, are located within malaria-prone areas and fall under active surveillance and control measures for malaria vectors, enabling proper breeding conditions for *Anopheles* sp mosquitoes (Mahdalena et al., 2015). The surrounding areas are characterized by the presence of rice fields where agricultural pesticides are commonly employed for pest control. The use of these agricultural pesticides can serve as a contributing factor to the resistance observed in *Anopheles* mosquitoes (Permenkes RI, 2017). Previous research has indicated that *Anopheles* mosquitoes in several districts in Africa exhibited potential resistance, which was closely associated with the use of agricultural pesticides (Reid & McKenzie, 2016).

The distribution of potential mosquito habitats in Banjarnegara Regency encompasses two research village locations, namely Gunungjati Village and Lebakwangi Village. In total, nine collection locations were selected for mosquito sampling, and all of them (100%) were in close proximity to potential breeding sites and residential areas. The buffer zone within a distance of <200 meters represents the effective range of the normal flight range of mosquitoes in search of blood sources from mosquito breeding sites. On the other hand, the zone beyond 500 meters from



potential breeding areas is influenced by wind factors. Notably, the residents' houses were observed to utilize household-scale insecticides in various forms such as sprays, burns, electric devices, and insecticide-treated nets. The analysis of the results reveals the existence of potential breeding habitats for *Anopheles* sp mosquitoes. Figure 3 illustrates the ability of mosquitoes to fly varying distances in Gunungjati and Lebakwangi Villages. The buffer zones within 1,000-2,000 meters from the breeding sites in these villages demonstrate their interconnectedness, potentially facilitating the movement, migration, and cross-breeding of mosquitoes across village boundaries. Moreover, the presence of potential breeding habitats for *Anopheles* sp mosquitoes within a distance of <500 meters in Gunungjati and Lebakwangi Villages indicates their close proximity to each other. Additionally, within the 1,000-2,000 meter buffer zone, brooding locations extend beyond the boundaries of individual villages or regions, potentially allowing for the distribution and movement of mosquitoes between adjacent areas. These findings suggest the potential for the dispersion of *Anopheles* mosquitoes from areas categorized as exposed to insecticides to areas categorized as not exposed to insecticides.

The observed resistance condition in areas not exposed to insecticides in the research villages was primarily due to their direct adjacency (distance < 2,000 meters) to areas with insecticide exposure. Spatial analysis, using buffer zones to assess the relationship between potential breeding habitats, residential houses/settlements, and paddy fields in terms of mosquito flight ability, indicates that the flight distance of female *Anopheles* sp mosquitoes from breeding sites is influenced by their preference for feeding sources for egg development. According to the theory, *Anopheles* sp mosquitoes are typically found within a distance of up to 2 km from their food sources, but strong winds can facilitate their flight for distances exceeding 30 km (Kazwaini, 2006; Service & Townson, 2002). Research conducted in East Sumba District-NTT also suggested that within a radius of 0-2,000 meters from breeding sites, the transmission of malaria occurs more rapidly (Willa & Kazwaini, 2015). The research villages included in this study are located in malaria receptive areas and are



actively monitored and controlled for malaria vectors. Malaria receptive areas possess the necessary conditions for *Anopheles* sp mosquitoes to breed effectively (Mahdalena et al., 2015).

Furthermore, areas with similar characteristics and borders have the potential for breeding, which aligns with the theory that areas more than 20 km apart should not be assumed to have the same resistance patterns. This situation facilitates the spread of malaria due to the presence of potential mosquito breeding habitats in proximity to human settlements. The buffer analysis captures the depiction of the normal flight range of mosquitoes, up to 2,000 meters, between the research village locations, enabling the ease of movement of resistant mosquitoes to areas not exposed to insecticides and their potential cross-breeding with susceptible mosquito populations. Spatial research on breeding sites and their distribution in surrounding areas has revealed a correlation with the occurrence of malaria transmission cases (Purnawati, 2016; Wijayanti, 2012).

Previous studies have indicated that settlements within close proximity to breeding areas result in higher densities of *Anopheles* sp mosquitoes within households (Kazwaini, 2006; Sari, 2018; Sucipto, 2014; Tindige et al., 2018; Willa & Kazwaini, 2015). Agriculture plays a vital role in the economy and livelihoods of the people in Banjarnegara Regency, creating a favorable environment for mosquito vectors to breed. In the research area of Gunungjati and Lebakwangi Villages, the conversion of land into salak gardens, with water ditches for irrigation, was observed. Land transformation due to human activities can shape and influence the presence of vector breeding sites and their distribution (Vanwambeke et al., 2007). The research area also encompasses rice fields where pesticides are commonly used for insect and plant pest control. The use of agricultural pesticides can contribute to the occurrence of resistance in *Anopheles* mosquitoes (Permenkes RI, 2017).

Previous research has indicated that tested *Anopheles* mosquitoes in several districts in Africa exhibited high resistance potentially linked to the use of agricultural pesticides (Reid & McKenzie, 2016). The uncontrolled use of pesticides in agricultural



areas creates selection pressure on the malaria vector population, leading to the evolution of insecticide resistance in malaria vectors (Matowo et al., 2020). The findings from this research can inform malaria vector control measures. The interpretation of test results based on threshold values for each type of insecticide can guide decision-making. Mosquitoes that survive beyond the threshold, representing a proportion of the mosquito population, indicate a certain level of resistance (Brogdon & Chan, 2010).

The Centers for Disease Control and Prevention (CDC) testing of bioassay bottles coated with a single insecticide provides information on the resistance status to specific insecticides in adult mosquitoes. Areas suspected of resistance to an insecticide can still continue with a control program, with a maximum application of six times (2-3 years). Furthermore, additional investigative tests should be conducted if single insecticide testing indicates suspected resistance. Additional testing at 5x and 10x concentrations can help understand the mechanisms and distribution of resistance. Additionally, it allows for the identification of areas where resistance occurs most intensively, thereby preserving the effectiveness of insecticides for malaria vector control programs and operational factors. Once resistance is detected, it serves as a reference for identifying other effective insecticide compounds not affected by cross-resistance (Brogdon & Chan, 2010).

Conclusion and Recommendation

The results of testing the resistance status of *Anopheles* sp mosquitoes in the categories of insecticide exposure and non-exposure (IRS) in Banjarnegara District showed no statistically significant difference in resistance to bendiocarb (Ficam 80 WP) 12.5 µg and lambda-cyhalothrin (Icon 100 CS) 12.5 µg. This lack of difference in resistance status can be attributed to the close proximity of potential breeding habitats in residential areas, where the community extensively uses mosquito control measures and pesticides in agricultural land. The resistance status of *Anopheles* sp mosquitoes



in areas without insecticide exposure is likely due to their adjacency to areas with insecticide exposure. This proximity creates a high potential for the movement and crossbreeding of resistant mosquitoes between the two locations, resulting in the production of resistant offspring. To ensure effective control measures, it is necessary to monitor the resistance status to insecticides in areas with active malaria transmission at an early stage. The use of insecticides such as lambda-cyhalothrin (100 CS) 12.5 μ g and bendiocarb (80 WP) 12.5 μ g, which have shown resistance in single tests, should be rotated after six applications (maximum of three years) with other insecticides that have different modes of action. By implementing a rotation strategy, the development of resistance can be slowed down, as different insecticides with varying killing power will be used. This approach helps to maintain the effectiveness of insecticides in controlling malaria vectors and reduces the risk of further resistance development.



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