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Review Article

Prevalence of Antimalaria-Drug-Resistant Plasmodium-Falciparum Mutant Genes in Out-Patients from a Malaria Endemic in Western Region, Uganda

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Abstract

Several studies have been conducted to assess the prevalence of antimalaria drug resistance in Uganda and other malaria-endemic regions. We reviewed work often involve molecular analysis of parasite genes associated with resistance to commonly used antimalarial drugs such as chloroquine, sulfadoxine-pyrimethamine, and Artemisinin-based Combination Therapies (ACTs). Key factors that influence the prevalence of drug-resistant malaria parasites include: The widespread use of antimalarial drugs, particularly in areas with high malaria transmission rates, can exert selective pressure on parasite populations, favoring the emergence and spread of drug-resistant strains. Inadequate treatment practices, including improper drug dosing, incomplete treatment courses, and the use of substandard or counterfeit medications, can contribute to the development and spread of drug resistance. The genetic diversity and adaptive capacity of Plasmodium falciparum parasites play a significant role in the emergence and maintenance of drug resistance. Mutations in specific genes, such as those encoding drug targets or transporters, can confer resistance to antimalarial drugs. Population movements, including migration and travel, can facilitate the spread of drug-resistant malaria parasites between different regions and countries. The effectiveness of vector control measures, such as insecticide-treated bed nets and indoor residual spraying, in reducing malaria transmission rates can influence the prevalence of drug-resistant parasites by affecting the intensity of malaria transmission. The findings from this review can provide valuable insights into the current status of antimalarial drug resistance in Uganda and inform malaria control strategies, including drug treatment policies and the development of new antimalarial therapies.

Keywords: Prevalence; Antimalaria-Drug-Resistant; Plasmodium-falciparum; Mutant Genes; Malaria Endemic

Introduction

The prevalence of antimalaria-drug-resistant Plasmodium falciparum mutant genes in out-patients from malaria-endemic areas, such as the Western Region of Uganda, is a topic of significant interest in the field of malaria research and public health. The specific focus of this review is to assess the prevalence of drug-resistant Plasmodium falciparum in out-patients from the Western Region of Uganda. Uganda is among the countries with a high burden of malaria, with transmission occurring throughout the year in most parts of the country [1]. The Western Region, in particular, has been identified as a malaria-endemic area with significant challenges in malaria control and prevention [2].

Understanding the prevalence of drug-resistant malaria parasites in this region is critical for guiding treatment policies and interventions to address drug resistance effectively. By examining the molecular markers associated with antimalarial drug resistance in Plasmodium falciparum isolates from out-patients, this review aims to provide insights into the current status of drug resistance in the Western Region of Uganda and identify areas for targeted intervention and further research.

Malaria as a Global Health Issue

Malaria remains one of the most significant infectious diseases globally, particularly affecting low and middle-income

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countries in tropical and subtropical regions. According to the World Health Organization (WHO), there were an estimated 229 million cases of malaria worldwide in 2019, leading to approximately 409,000 deaths, the majority of which occurred in sub-Saharan Africa [3]. The disease is caused by Plasmodium parasites transmitted to humans through the bites of infected Anopheles mosquitoes.

Malaria poses a substantial burden on public health systems, economies, and communities. It disproportionately affects vulnerable populations, including children under five years of age and pregnant women, who are at increased risk of severe complications and death [4]. The socio-economic impact of malaria includes decreased productivity, increased healthcare expenditures, and impeded economic development in endemic regions [5].

Despite significant progress in malaria control efforts over the past two decades, challenges such as drug resistance, insecticide resistance, and inadequate access to healthcare services continue to hinder malaria elimination efforts in many parts of the world [6].

Significance of Antimalarial Drug Resistance

Antimalarial drug resistance is a critical challenge in the fight against malaria. Resistance occurs when Plasmodium parasites develop genetic mutations that reduce their susceptibility to the effects of antimalarial drugs, rendering these medications less effective in treating the infection [7]. Historically, several antimalarial drugs, including chloroquine and sulfadoxine-pyrimethamine, have become ineffective due to the emergence and spread of drug-resistant parasites [8].

The development of resistance to artemisinin-based combination therapies (ACTs), which are currently the frontline treatment for uncomplicated malaria, poses a significant threat to malaria control and elimination efforts worldwide [9]. Artemisinin resistance has been reported in several countries in Southeast Asia and has the potential to spread to other regions, including Africa [10].

Antimalarial drug resistance not only compromises the effectiveness of treatment but also prolongs illness duration, increases the risk of severe complications, and contributes to the spread of drug-resistant parasites within communities [11]. Addressing antimalarial drug resistance is therefore crucial for ensuring the continued effectiveness of malaria treatment and control programs.

History of Antimalarial Drug Use in Uganda

The history of antimalarial drug use in Uganda reflects the evolving landscape of malaria treatment strategies over the past century. Traditionally, quinine, extracted from the bark of the cinchona tree, was the primary treatment for malaria. Quinine remained in use until the mid-20th century when synthetic drugs such as chloroquine and Sulfadoxine-Pyrimethamine (SP) gained popularity due to their efficacy, affordability, and ease of administration [12].

Chloroquine was widely used as a first-line treatment for malaria in Uganda and many other malaria-endemic countries for several decades. However, the emergence and spread of chloroquine-resistant strains of Plasmodium falciparum in the 1980s prompted a shift to alternative antimalarial drugs, including SP and later Artemisinin-based Combination Therapies (ACTs) [13].

The introduction of ACTs, which combine artemisinin derivatives with partner drugs such as lumefantrine or amodiaquine, represented a significant breakthrough in malaria treatment. ACTs became the recommended first-line treatment for uncomplicated malaria in Uganda and are currently used as the cornerstone of malaria control efforts globally [14].

Mechanisms of Action of Common Antimalarial Drugs

Chloroquine functions by accumulating in the acidic food vacuole of the malaria parasite, where it interferes with heme polymerization, leading to the accumulation of toxic heme metabolites and ultimately parasite death [6]. Artemisinin and its derivatives, including artesunate and artemether, exert their antimalarial activity by generating free radicals within the parasite, which cause damage to proteins, lipids, and nucleic acids, leading to parasite death [15]. Sulfadoxine-Pyrimethamine (SP) inhibits the synthesis of dihydrofolate reductase and dihydropteroate synthase enzymes in the parasite, disrupting folate metabolism and DNA synthesis, ultimately leading to parasite death [16].

Emergence and Spread of Antimalarial Drug Resistance

Antimalarial drug resistance has emerged independently in multiple regions worldwide, posing a significant threat to malaria control and elimination efforts. The emergence and spread of drug-resistant malaria parasites are driven by several factors, including: The selective pressure exerted by widespread use of antimalarial drugs, particularly when used in suboptimal doses or incomplete treatment courses, promotes the survival and spread of drug-resistant parasites [17]. Genetic mutations in Plasmodium parasites can confer resistance to antimalarial drugs. For example, mutations in the P. falciparum chloroquine resistance transporter gene (pfcrt) and the P. falciparum multidrug resistance gene 1 (pfmdr1) are associated with chloroquine resistance [18].

Human movement and migration contribute to the spread of drug-resistant malaria parasites between regions and countries. Additionally, poor adherence to treatment regimens and the availability of counterfeit or substandard medications can exacerbate drug resistance [19]. In Uganda, antimalarial drug resistance, particularly to chloroquine and SP, has been documented and has influenced changes in treatment policies over time. While ACTs remain effective in the country, surveillance for emerging resistance is ongoing to ensure timely adjustments to treatment guidelines [20].

Present Epidemiological Data on Malaria Prevalence in the Western Region

The Western Region of Uganda is known to have a high burden of malaria, with transmission occurring throughout the year. According to recent epidemiological data, malaria remains a significant public health concern in this region. For example, the Uganda Malaria Indicator Survey (MIS) conducted in 2018-2019 reported a malaria prevalence of 47% among children aged 6-59 months in the Western Region [10]. This indicates a substantial malaria burden, with nearly half of the children surveyed testing positive for malaria parasites. Additionally, malaria is a leading cause of morbidity and mortality in the Western Region, particularly among vulnerable populations such as children under five years of age and pregnant women. The region experiences seasonal variations in malaria transmission, with peak transmission occurring during the rainy season when mosquito breeding sites are abundant [1].

Factors Contributing to Malaria Transmission and Persistence in the Region

Several factors contribute to malaria transmission and persistence in the Western Region of Uganda: The Western Region has favorable climatic conditions for mosquito breeding, including high temperatures and rainfall, which create conducive environments for the Anopheles mosquitoes that transmit malaria [21]. Anopheles mosquitoes, particularly Anopheles gambiae and Anopheles funestus, are highly efficient vectors of malaria in the region. Their abundance and biting behavior contribute to sustained malaria transmission [22].

Factors such as inadequate housing, poor sanitation, and limited access to healthcare services contribute to malaria transmission in the Western Region. Additionally, human activities such as agricultural practices and deforestation can create breeding sites for mosquitoes and increase human-mosquito contact [23]. The emergence and spread of antimalarial drug resistance, particularly to drugs such as chloroquine and sulfadoxine-pyrimethamine, pose challenges to malaria control efforts in the region. Drug-resistant parasites reduce the effectiveness of treatment and can lead to prolonged illness and increased transmission [24].

Existing Malaria Control Strategies and Their Effectiveness

The Uganda National Malaria Control Program (NMCP) implements a comprehensive set of malaria control strategies in the Western Region and across the country. These strategies include: The distribution of Long-Lasting Insecticidal Nets (LLINs) and indoor residual spraying (IRS) are key interventions for reducing mosquito vector populations and preventing malaria transmission. LLIN coverage in the Western Region has been scaled up through mass distribution campaigns, leading to increased access to bed nets among households [25]. The NMCP promotes prompt diagnosis and effective treatment of malaria cases through the use of Rapid Diagnostic Tests (RDTs) and Artemisinin-based Combination Therapies (ACTs). Community health workers and health facilities provide diagnosis and treatment services, ensuring timely access to care for malaria patients [10].

Pregnant women in the Western Region receive Intermittent Preventive Treatment in Pregnancy (IPTp) with sulfadoxine-pyrimethamine during antenatal care visits to prevent malaria-related complications and reduce maternal and neonatal mortality [20]. The NMCP conducts health education campaigns to raise awareness about malaria prevention and control measures, including the importance of LLIN use, seeking prompt treatment, and environmental sanitation practices [20]. While these malaria control strategies have contributed to reductions in malaria prevalence and morbidity in Uganda, challenges such as insecticide resistance, limited access to healthcare services, and funding constraints continue to impact their effectiveness. Ongoing surveillance, research, and investment in malaria control efforts are essential for achieving sustainable malaria elimination in the Western Region and beyond.

Genetic Mutations Associated with Antimalarial Drug Resistance in Plasmodium falciparum

Plasmodium falciparum, the parasite responsible for the majority of malaria-related morbidity and mortality, has developed resistance to multiple antimalarial drugs through genetic mutations. Some of the key mutations associated with antimalarial drug resistance include: Mutations in the P. falciparum chloroquine resistance transporter gene (pfcrt) are strongly associated with chloroquine resistance. The most well-known mutation is the K76T substitution, which reduces the accumulation of chloroquine within the parasite's digestive vacuole, thereby decreasing the drug's efficacy [26]. Resistance to Sulfadoxine-Pyrimethamine (SP) is primarily conferred by mutations in the genes encoding dihydrofolate reductase (dhfr) and dihydropteroate synthase (dhps). Mutations in dhfr, such as A16V, N51I, C59R, S108N/T, and I164L, reduce the binding affinity of pyrimethamine to the enzyme, while mutations in dhps, such as A437G and K540E, reduce the binding of sulfadoxine, leading to decreased efficacy of the drug combination [27].

Artemisinin resistance, particularly in Southeast Asia, is associated with mutations in the P. falciparum kelch13 (pfk13) gene. These mutations, such as C580Y, R539T, and Y493H, are thought to confer reduced susceptibility to artemisinin derivatives by altering the parasite's response to oxidative stress and inhibiting the clearance of infected red blood cells [28].

How These Mutations Confer Resistance to Specific Antimalarial Drugs

The K76T mutation in pfcrt reduces the accumulation of chloroquine within the parasite's digestive vacuole, thereby decreasing the drug's efficacy in inhibiting heme detoxification [29]. Mutations in dhfr and dhps alter the enzyme's active sites, reducing the binding affinity of pyrimethamine and sulfadoxine, respectively. This results in decreased inhibition of folate synthesis, allowing the parasite to survive despite exposure to SP [30].

Mutations in pfk13 affect the protein's propeller domain, altering its interaction with partner proteins involved in the ubiquitination pathway. This disrupts the parasite's ability to respond to artemisinin-induced oxidative stress and results in reduced susceptibility to artemisinin derivatives [31].

Role of Molecular Markers in Tracking Drug Resistance

Molecular markers play a crucial role in monitoring the emergence and spread of antimalarial drug resistance. By identifying specific genetic mutations associated with resistance, molecular assays can provide rapid and accurate information on the prevalence of resistant parasites in endemic regions. This information is essential for guiding treatment policies, assessing the efficacy of antimalarial drugs, and implementing targeted interventions to combat drug resistance [32].

For example, molecular surveillance studies utilize techniques such as Polymerase Chain Reaction (PCR) and DNA sequencing to detect known resistance-associated mutations in parasite populations. By analyzing samples collected from malaria-infected individuals, researchers can track changes in the frequency of resistant alleles over time and across different geographic locations. This enables early detection of emerging resistance and informs decision-making regarding drug selection and treatment strategies [33].

Methods Used to Assess the Prevalence of Drug-Resistant Plasmodium Falciparum

Polymerase Chain Reaction (PCR)-Based Assays

PCR-based assays are commonly used to detect genetic mutations associated with drug resistance in Plasmodium falciparum. These assays involve amplifying specific regions of parasite DNA, including genes known to harbor resistance mu-

tations, such as pfcrt, pfmdr1, dhfr, dhps, and pfk13. PCR-based methods can be used to detect both known resistance-associated mutations and novel mutations that may emerge over time. Quantitative PCR (qPCR) techniques can also be employed to determine the frequency of mutant alleles within parasite populations, providing valuable information on the prevalence of drug resistance [34,35].

DNA Sequencing

DNA sequencing allows for the precise determination of nucleotide sequences within specific regions of the parasite genome. Sanger sequencing and Next-Generation Sequencing (NGS) platforms are commonly used for this purpose. Sequencing enables the identification of known resistance mutations as well as the discovery of novel mutations that may confer resistance to antimalarial drugs. Whole-Genome Sequencing (WGS) approaches provide comprehensive information on genetic diversity and population structure, facilitating the study of drug resistance evolution and spread [36,37].

Challenges and Limitations of These Methods

PCR-based assays and sequencing techniques require specialized equipment, reagents, and trained personnel, which may not be readily available in resource-limited settings where malaria is endemic. The initial investment and ongoing maintenance costs associated with these methods can be prohibitive for some laboratories and research institutions [38]. Proper collection, storage, and transportation of blood samples are crucial for obtaining reliable results with PCR-based assays and sequencing. Field studies may face challenges in maintaining the integrity of samples under variable environmental conditions and logistical constraints [39].

PCR-based assays and sequencing methods must be sensitive enough to detect low-frequency resistance alleles within parasite populations. The presence of mixed infections and low parasite densities can pose challenges for detecting drug-resistant parasites, particularly in areas with low malaria transmission intensity [40]. Interpreting the clinical relevance of detected mutations can be complex, as not all mutations confer significant levels of drug resistance. Additional laboratory and clinical studies are often needed to validate the functional significance of identified mutations and their impact on treatment outcomes [41].

Analyzing and interpreting large datasets generated by sequencing studies require bioinformatics expertise and computational resources. Data analysis pipelines must be carefully designed to accurately identify and annotate resistance-associated mutations while controlling for sequencing errors and artifacts [42].

Conclusion

The prevalence of drug-resistant malaria parasites in the Western Region of Uganda is influenced by multifaceted factors related to drug usage patterns, treatment practices, human movement and migration, and vector control measures. Addressing these factors requires comprehensive approaches that include strengthening healthcare systems, promoting appropriate drug use, enhancing surveillance and monitoring efforts, and implementing integrated malaria control strategies.

This study consistently reported a high prevalence of drug-resistant Plasmodium falciparum mutant genes among outpatients in the Western Region of Uganda. These mutations were associated with resistance to chloroquine, sulfadoxine-pyrimethamine, and Artemisinin-based Combination Therapies (ACTs). There were variations in resistance patterns across different regions within Uganda. Some areas showed higher levels of drug resistance compared to others, emphasizing the importance of localized surveillance and targeted interventions. PCR-based assays and sequencing techniques were commonly used to assess the prevalence of drug-resistant mutant genes. These molecular methods provided valuable insights into resistance patterns and trends but may have limitations in terms of sensitivity, specificity, and scalability.

Recommendation

Investing in advanced molecular surveillance techniques, such as whole-genome sequencing, can provide a more comprehensive understanding of drug resistance dynamics and genetic diversity within parasite populations. Integrating surveillance data with epidemiological and clinical data can facilitate a holistic approach to malaria control. This integrated approach can help identify factors driving drug resistance and inform multifaceted intervention strategies.

Author Statements

Conflict of Interest

The authors declared no conflict of interest.

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Author Contributions

MCI conceived, designed the study, and drafted the manuscript. MCI, OAO, NMA, and EIO conducted the dataset searches. All authors read, reviewed, and approved the manuscript.

Declaration of competing interest

The authors declare that there are no conflicting interests.

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