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STATUS OF ARTEMISININ RESISTANCE-CONFERRING *PLASMODIUM FALCIPARUM* MALARIA KELCH-13 R561H POLYMORPHISM IN MUTASA DISTRICT, ZIMBABWE

BY

NOBERT MUDARE

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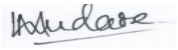
Abstract

Malaria is a major public health problem, with 228 million cases and 405,000 deaths in 2018. Mutations in the PfKelch13 propeller domain constitute the molecular basis of artemisinin resistance. These mutations are suspected to reduce function of the drug target, PfKelch13, rendering the parasite resistant. The aim of the current study was to determine prevalence of artemisinin resistance-conferring *P. falciparum* Kelch 13 R561H mutant parasites and their relation to ACT treatment failure in Mutasa District from April 2021- June 2021. An unmatched case-control study was conducted in Mutasa District clinics (St Peters, Chinaka and Chisuko). Participants with a malaria RDT positive test result was enrolled after consenting. A Dried Blood spot sample was collected and transported to Africa University Laboratories for genotyping of the Kelch13 R561H mutations in *P. falciparum* parasites. ACT drugs were bought in Mutasa communities and Mozambique boader with Zimbabwe and were tested using the Guo et al. (2016) Dipstick test for detecting fake/ substandard ACT drugs. A total of 82 participants were enrolled with 32 cases and 50 controls. 87 DBS from the lab repository collected in 2003 when ACT were also genotyped. A Bivariate analysis of the risk factors for suspected malaria treatment failure was done. The risk factors analysed comprised ACT readily available($p=0.523$), test for malaria before getting ACT($p=0.403$), patient received health education regarding ACT adherence($p=0.536$), Completed ACT course($p=0.411$), community campaigns on ACT use($p=0.240$), experienced side effects after taking ACT($p=0.418$), received counselling on ACT use($p=0.614$) and ever bought ACT from vendors($p=0.912$). All these risk factors were not associated with suspected malaria treatment failure. Laboratory genotyping of the K13 R561H markers, 4.3% of the analysed samples were K13R561H mutant type parasites while 42.0% were K13R561H wild type. ACT drug quality test showed that 7.1% of the ACTs bought along the border were fake or false. The suspected malaria treatment failure was attributed to the presence of the K13R561H mutations. Fake/ falsified antimalarial ACT drugs were observed in Mutasa District. None of the suspected malaria treated failure risk factors were associated with malaria treatment failures in Mutasa District possibly due to small sample size achieved in current study. The newly lab developed assay K13R561H SNP assay can be used for monitoring ACT drug resistance and inform policy, subject to further field validations.

Declaration

I declare that this research is my original work except where sources have been cited and acknowledged. The work has never been submitted, nor will it ever be submitted to another university for the award of a degree.



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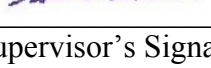
Sungano Mharakurwa



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Main Supervisor's Full Name Main Supervisor's Signature (Date)

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Dedication

This work is dedicated to my dear family wife and two children, who gave me the reason and anchor to persevere when I had valid reasons to give up. I also dedicated this work to the Almighty God for His extraordinary grace that pulled me through when it seemed impossible.

List of Acronyms and Abbreviations

NMCP	National Malaria Control Programme
DEHO	District Environmental Health Officer

EHT	Environmental Health Technician
ACT	Artemisinin-based combination therapy
CDC	Centre for Disease Control and Prevention
DDT	Dichlorodiphenyltrichloroethane
DHS	Demographic health survey
IVM	Integrated vector management

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CHAPTER 1 INTRODUCTION

1.1 Introduction

Malaria represents a major public health issue in the tropics, with an estimated 228 million cases and 405,000 deaths in 2018 globally (White, 2014; WHO, 2019). Of increasing concern is *P. falciparum* resistance to Artemisinin combination therapy (ACT) derivatives, used worldwide as the core components of Artemisinin-based combination therapies (ACTs) (Menard, 2019). ACT resistance, characterized by delayed *P. falciparum* clearance following treatment with Artemisinin mono-therapy or an ACT (Ashley et al., 2014; Dondorp et al., 2009) and is now widespread in the Greater Mekong Subregion (GMS), which consists of Cambodia, Thailand, Vietnam, Myanmar and Laos (Amato et al., 2018; Imwong et al., 2017). Resistance to the partner drugs piperaquine and Mefloquine is also now common in the GMS, causing high rates of ACT treatment failure (Hamilton et al., 2019; Pluijm et al., 2019).

The appearance of ACT resistance parasites in Africa would pose a major public health threat. Resistance to the former first-line antimalarial Chloroquine first arose in the GMS in the 1960s before spreading to Africa. Resistance to Pyrimethamine (used in association with Sulfadoxine) followed shortly thereafter (Blasco et al., 2017). The lost clinical efficacy of these compounds is suspected to have contributed to millions of additional malaria deaths in young African children in the 1980s (Murray et al., 2012). In addition to the risk of imported resistance, the likelihood of resistance emerging locally in Africa has increased in areas where control measures have reduced the disease transmission intensity (Huang et al., 2013). The resulting attenuation in naturally

acquired human immunity can increase the frequency of symptomatic infections and the need for treatment, while decreasing parasite genetic diversity and reducing competition between sensitive and resistant parasites (Scott et al., 2018).

To date, the efficacy of ACTs has remained high outside Southeast Asia (SEA) (Conrad et al., 2019). Early detection of resistance provides the best chance of minimizing its lethal impact. Mutations in the Pfkelch13 propeller domain (PF3D7_1343700) constitute the primary determinant of ACT resistance (Ashley et al., 2014, Ariey et al., 2014 &Straimer et al., 2015). These mutations are suspected to reduce Pfkelch13 function, which is required for parasite-mediated endocytosis of host hemoglobin in the newly invaded intra-erythrocytic ring stages (Birnbaun et al., 2020; Yang et al, 2021). Pfkelch13 C580Y is the most widespread allele in SEA (Hamilton et al, 2019; Imwong et al., 2017) and has recently been detected in Guyana and Papua New Guinea (Mathieu et al., 2020; Miotto et al., 2019).

In Africa, slow-clearing infections after ACT treatment have been observed at frequencies of 50% of the polymorphisms present in only a single *P. falciparum* infection. The most frequent Pfkelch13 mutation in Africa was A578S, which did not confer ACT resistance in vivo or in vitro (Menard et al., 2016). No synonymous Pfkelch13 mutations associated with delayed parasite clearance or day 3 positivity (day 3+) in the GMS (F446I, Y493H, R539T, I543T, P553L, R561H, P574L, C580Y, A675V) have been reported, in African parasites (Ocan et al., 2019; WWARN, 2019)

1.2 Background to the study

Plasmodium falciparum is the most widespread *Plasmodium* species and is responsible for the majority of severe forms and deaths related to malaria in sub-Saharan Africa. *P. falciparum* has succeeded in developing resistance mechanisms against almost all existing antimalarial drugs, which is a major threat to malaria control worldwide. The high level of *P. falciparum* resistance to Chloroquine (CQ) and then to Sulfadoxine-Pyrimethamine (SP) has led endemic countries to change their antimalarial drug policy of uncomplicated malaria treatment. Artemisinin-based Combination Therapies (ACTs) have been adopted as first-line treatment of uncomplicated malaria in 2005 (Swarthout et al., 2006). With the discovery of several *P. falciparum* genes involved in antimalarial drug resistance, molecular markers have become a precious tool in resistance surveillance (Cui et al., 2015;
Vestergaard et al., 2007).

Mutations in the propeller domain of *P. falciparum* Kelch 13 gene (pfk13) have been identified as associated with *in vivo* delayed parasite clearance and *in vitro* artemisinin resistance in ring stage survival Assay (RSA) (Ashley et al., 2014; Ariey et al., 2014). These mutations spread into the Greater Mekong Sub-region (GMS) of Southeast Asia (Menard et al., 2016; WWARN, 2019) and have been recently classified in validated markers of ART-R (F446I, N458Y, M476I, Y493H, R539T, I543T, P553L, R561H and C580Y) and candidate/associated markers of ACT-Resistant (P441L, G449A, C469F, A481V, P527H, N537I, G538V, V568G, P574L, F673I, A675V) (WHO, 2018).

In Sub-Saharan Africa, there has been no evidence for emergence of Pf ART-R until now. Mutations detected in Africa have not been associated to ACT-Resistance like those reported in Southeast Asia (Kamau et al., 2015, Menard et al., 2016, Taylor et al, 2015; WWARN, 2019). Some mutations associated with slow parasite clearance detected in Africa have been reported with very low relative frequency (Ikeda et al., 2018, Kakolwa et al., 2018 & Tacoli et al., 2016). The spread of ACT-Resistance from the GMS to Africa, like previously happened with other antimalarial drugs (Mita et al., 2011) may be a major obstacle for malaria control and elimination around the World.
In Zimbabwe, like in almost all Sub-Saharan countries, there are currently no data of the validated ACT resistance and this underscores the importance of the current study.

However, the Pfkelch 13 R561H mutation which has been described to be associated with delayed clearance has been reported in a previous study in 21 isolates in Rwanda (Uwimana et al., 2020). Mutasa District has been experiencing suspected cases of malaria treatment failure, suggestive of potential *P. falciparum* ACT resistance, for which there are currently no data in Zimbabwe. These malaria treatment failures were observed in the following five (5) Health facilities which are Chinaka, St Peters, Chisuko, Katiyo and Sagambe. The current study investigated the Artemisinin-based combination therapy (ACT) resistance-associated pfk13 R561H-propeller polymorphisms in clinical isolates of *P. falciparum* collected in Mutasa.

1.3 Statement of the problem

Artemisinin-based combination therapy (ACT) is recommended as first-line treatment of uncomplicated *Plasmodium falciparum* malaria in Zimbabwe. The treatment **must** ensure the rapid and full elimination of *Plasmodium* parasites from a patient's

bloodstream in order to prevent an uncomplicated case of malaria from progressing to severe disease or death. Malaria treatment failure cases that came after the initial administration of ACTs in 2020 have been recorded at five clinics in Mutasa along the border with Mozambique. Therefore, there is a need to assess the status of artemisinin-based combination therapy resistance –conferring *Plasmodium falciparum* malaria *Kelch-13 R561H* polymorphism in Mutasa.

Table 1 Malaria treatment failure cases recorded after the initial administration of ACTs at clinics in Mutasa 2020

Health facility	Treatment failures	Percentage
Chinaka	21/4089	0.5
St Peters	17/3958	0.4
Chisuko	19/2273	0.8
Katiyo	15/2873	0.5
Sagambe	9/1419	0.6
Total	81/14612	0.6

Table 1.1 Mutasa ACT treatment failure data (Zimbabwe DHIS2, 2020)

1.4 Research Objectives

1.4.1 Broad Objectives

i. To determine prevalence of Artemisinin resistance-conferring *P. falciparum* Kelch 13 R561H mutant parasites and their relation to ACT treatment failure in Mutasa District

1.4.2 Specific Objectives

i. To determine the prevalence of *P. falciparum* malaria ACT treatment failure cases and the Pfkelch13 R561H mutation in Mutasa, Manicaland in Zimbabwe

ii. To identify the risk factors of ACT treatment failure among malaria cases presenting at Chinaka, St Peters and Chisuko clinic in Mutasa Manicaland, Zimbabwe

iii. To evaluate the quality of ACTs obtained from community vendors.

1.5 Research questions

i. What is the prevalence of *P. falciparum* malaria ACT treatment failure cases and the Pfkelch13 R561H mutation in Mutasa, Manicaland in Zimbabwe?

ii. What are the risk factors of *P. falciparum* malaria ACT treatment failure cases and the Pfkelch13 R561H mutation among malaria cases presenting at Chinaka, St Peters and Chisuko clinic, Manicaland, Zimbabwe?

iii. What is the quality of Artemisinin-based combination therapy (ACT) drugs obtained from community vendors in Mutasa Manicaland Zimbabwe?

1.6 Justification

Research on what is causing ACT treatment failure is non-existent in Zimbabwe. The study provided updates on the global situation of artemisinin drug resistance in Zimbabwe. The study also provided updates on the most recent tools for monitoring drug efficacy and drug resistance. The information on therapeutic efficacy generated should be shared among malaria programme managers in order to provide the best possible advice to ministries of health.

1.7 Delimitations of the study

- The study focused on *P. falciparum* genotyping of the **Kelch 13** mutant markers, patients at Chinaka, St Peters and Chisuko clinics only.

Limitations

The malaria burden was very low in Mutasa during the 2021 malaria season (from April 2021 to June 2021 study period).

CHAPTER 2: REVIEW OF RELATED LITERATURE

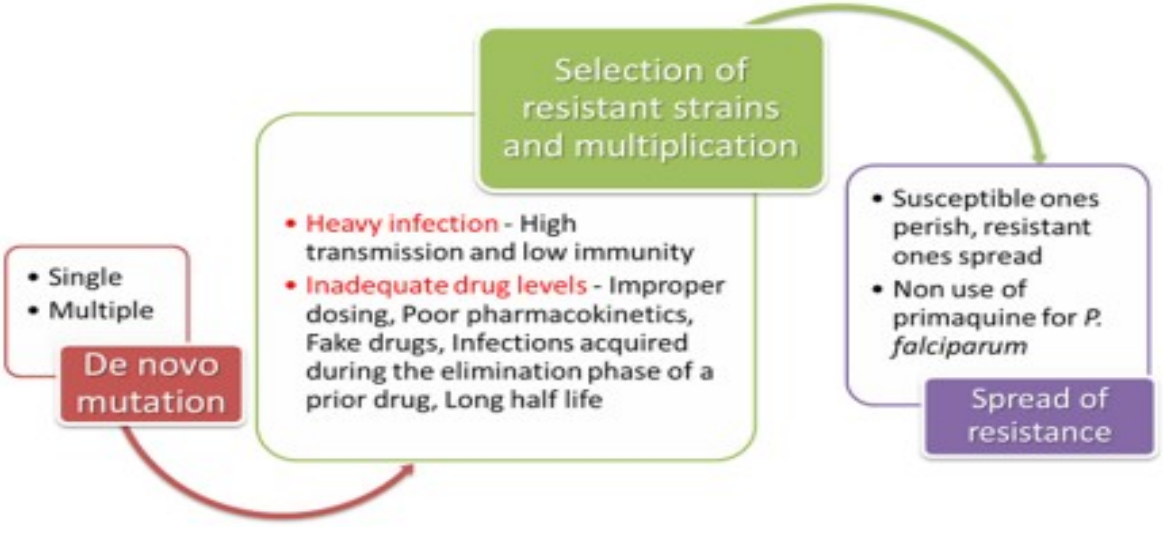
2.1 Introduction

This chapter reviews literature on the prevalence of *P. falciparum* malaria ACT treatment failure cases and the Pfkelch13 R561H mutation, risk factors of ACT treatment failure among malaria cases. Then will also look at quality of antimalarial drugs being used to treat malaria cases. Special focus is given to what other studies have reported as potential risk factors for ACT treatment failure and the PfKelch 13 R561H mutations development.

2.2 Conceptual Framework

The study conceptual framework was developed guided by the World Health Organization (WHO), 2010 antimalarial drug resistance spread as shown in the figure 1 below.

Fig. 2.1. Conceptual framework for antimalarial drug resistance spread. (WHO, 2010)



According to the framework, the malaria parasite is well known for its frequent, de novo mutations, mostly single, and sometimes multiple. In the presence of heavy infection and inadequate drug levels, the resistant mutations survive and propagate. Development of resistance requires a high grade of parasitemia, coupled with low or inadequate drug levels. Most cases of resistance have emerged out of SE Asia region. This region is known for low transmission and low immunity that lead to high parasitemia; it also has a long history of indiscriminate use of different antimalarial drugs.

In such low-transmission areas, most malaria infections are symptomatic, and therefore proportionally more people receive treatment, providing more opportunities for selection of resistant strains. One study also suggested that *P. falciparum* in South-East Asia has an inherent propensity to develop drug resistance through genetic mutation. Areas with very high transmission, such as Africa, appear to be less susceptible to the emergence of drug resistance. In these areas, infections are acquired repeatedly throughout life, resulting in partial immunity ("premunition"), that in turn controls the infection, usually at levels below those that cause symptoms. Asymptomatic infection and often non-availability of drugs in these areas mean that these patients do not receive antimalarial drugs, and hence the chances of development of resistance are lower (WHO, 2010).

Immunity acts by non-selectively eliminating blood-stage parasites, including the rare de novo resistant mutants, and also improves cure rates, even with failing drugs, thereby reducing the relative transmission advantage of resistant parasites. Furthermore, complex polyclonal infections in semi-immune people allow possible outbreeding of mutagenic resistance mechanisms or competition in the host or the mosquito between less-fit resistant strains and more-fit sensitive strains (WHO, 2010).

2.3 Significance of the conceptual framework

The conceptual framework highlights the key variables that can influence antimalarial treatment failure and spread of ACT resistance and these are discussed as below:

2.3.1 Prevalence of *P. falciparum* malaria ACT treatment failure cases and the Pfkelch13 R561H mutation.

Dondorp *et al*. (2009) found out that the overall median parasite clearance times for ACT drugs were 84 hours (interquartile range, 60 to 96) in Pailin and 48 hours (interquartile range, 36 to 66) in Wang Pha (P<0.001). Recrudescence confirmed by means of polymerase chain-reaction assay occurred in 6 of 20 patients (30%) receiving Artesunate monotherapy and 1 of 20 (5%) receiving Artesunate–Mefloquine therapy in Pailin, as compared with 2 of 20 (10%) and 1 of 20 (5%), respectively, in Wang Pha (P = 0.31). Ajay et al. (2013) in Nigeria reported three cases of early treatment failure due to possible Artemisinin-based combination therapy-resistant *Plasmodium falciparum* malaria. All cases showed adequate clinical and parasitological responses to quinine.

In other studies, Hodel et al. (2016) have shown that Artemisinin kill rates may have been substantially overestimated in previous modeling studies. The reasons for overestimation could be because (i) the parasite reduction ratio (PRR) (generally estimated to be 104) is based on observed changes in circulating parasite numbers, which generally overestimate the “true” PRR, which should include both circulating and sequestered parasites, and (ii) the third dose of Artemisinin at 48 hours targets exactly those stages initially hit at time zero, so it is incorrect to extrapolate the PRR measured over 48 hrs to predict the impact of doses at 48 hours and later.

In contrast, Mbah et al. (2015) showed that Dihydroartemisinin-Piperaquine (DP) is more effective in reducing malaria incidence of clinical episodes than Artemether-Lumefantrine (AL). DP should be considered as a replacement for AL as first-line treatment of uncomplicated malaria in highly endemic *P. falciparum* communities. However, Woodrow et al. (2017) found that artemisinins are the most rapidly acting of currently available antimalarial drugs. Artesunate has become the treatment of choice for severe malaria, and Artemisinin-based combination therapies (ACTs) are the foundation of modern *falciparum* malaria treatment globally. Their safety and tolerability profile is excellent. Unfortunately, *Plasmodium falciparum* infections with mutations in the 'K13' gene, with reduced ring-stage susceptibility to artemisinin, and slow parasite clearance in patients treated with ACTs, are now widespread in Southeast Asia (Imwong et al., 2017; Ashley et al., 2014).

In addition, evidence for the de novo emergence of Pfkelch13-mediated Artemisinin resistance in Rwanda has been recorded, potentially compromising the continued success of antimalarial chemotherapy in Africa (Uwimana et al., 2020).

2.3.2 Parasite fitness

Gimode et al. (2015) found that during serial passaging in the absence of the drug, the PQ-resistant parasite maintained low growth rates at day 7 pi (mean parasitaemia, 5.6% ± 2.3) relative to the wild-type (28.4% ± 6.6), translating into a fitness cost of resistance of 80.3%. The contrasting behavior of PQ- and LU-resistance phenotypes indicate that even for drugs within the same chemical class, resistance-conferred traits may vary on how they influence parasite fitness and virulence. Tyrrell et al. (2019) found that a clinically Artemisinin resistant parasite line that carries the wild-type form of kelch13

outcompeted all other parasites in their study. Furthermore, a kelch13 mutant line (E252Q) was competitively more fit without drug than lines with other resistance-associated kelch13 alleles, including the C580Y allele that has expanded to high frequencies under drug pressure in Southeast Asian resistant populations.

In related studies, Nair et al. (2018) reported that their fitness cost study reveals that kelch13 C580Y carries higher fitness costs (s [selection coefficient] 0.15 0.008 [1 standard error]) than R561H (s 0.084 0.005). Furthermore, R561H outcompetes C580Y in direct competition (s 0.065 0.004). In contrast, Bunditvorapoom et al. (2018) reported that artemisinin-resistant *P. falciparum* isolates from Cambodia manifested fitness loss, showing fewer progenies during the intra-erythrocytic developmental cycle. The loss in fitness was exacerbated under the condition of low exogenous amino acid supply. The resistant parasites failed to undergo maturation, whereas their drug-sensitive counterparts were able to complete the erythrocytic cycle under conditions of amino acid deprivation. These findings on fitness and protein homeostasis could provide clues on how to contain emerging artemisinin-resistant parasites.

However, Hott et al. (2015) demonstrated for the first time that Artemisinin resistance provides a fitness advantage that is selected for with infrequent exposure to drug, but is lost in the absence of exposure to artemisinin drugs. Artemisinin-resistant *P. falciparum* have a fitness advantage to survive and predominate in the population even in the face of infrequent exposure to artemisinin drugs. Furthermore, Woodrow et al. (2017) reported that the ring-stage activity of artemisinin is therefore critical for the sustained efficacy of ACTs; once it is lost, rapid selection of partner drug resistance and ACT failure are inevitable consequences.

Despite increased investment in regional control activities, ACTs are failing across an expanding area of the Greater Mekong sub region. Although multiple K13 mutations have arisen independently, successful multidrug-resistant parasite genotypes are taking over and threaten to spread to India and Africa. Stronger containment efforts and new approaches to sustaining long-term efficacy of antimalarial regimens are needed to prevent a global malaria emergency

2.3.3 Prevalence of *P. falciparum* malaria cases harboring the Pfkelch13 R561H mutation.

Ashley et al. (2014) reported that the median parasite clearance half-lives ranged from 1.9 hours in the Democratic Republic of Congo to 7.0 hours at the Thailand–Cambodia border. Slowly clearing infections (parasite clearance half-life >5 hours), strongly associated with single point mutations in the "propeller" region of the *P. falciparum* kelch protein gene on chromosome 13 (kelch13), were detected throughout mainland Southeast Asia from southern Vietnam to central Myanmar. The incidence of pretreatment and post-treatment gametocytemia was higher among patients with slow parasite clearance, suggesting greater potential for transmission. In western Cambodia, where Artemisinin-based combination therapies are failing, the 6-day course of antimalarial therapy was associated with a cure rate of 97.7% (95% confidence interval, 90.9 to 99.4) at 42 days.

In contrast, Patel et al. (2017) reported that no mutation was found in the K-13 propeller gene, while only one sample showed a mutant genotype for the PfATPase6 gene. The parasites remain sensitive to Artemisinin derivatives. However, Laurent et al, 2018

found that nineteen non synonymous K13 mutations were present in the pre-ACT samples (N = 64) compared with 22 in the post-ACT samples (N = 251).

Another study by Ariey et al. (2014) found that mutant K13-propeller alleles cluster in Cambodian provinces where resistance is prevalent, and the increasing frequency of a dominant mutant K13-propeller allele correlates with the recent spread of resistance in western Cambodia. Strong correlations between the presence of a mutant allele, in vitro parasite survival rates and in vivo parasite clearance rates indicate that K13-propeller mutations are important determinants of Artemisinin resistance. K13propeller polymorphism constitutes a useful molecular marker for large-scale surveillance efforts to contain Artemisinin resistance in the Greater Mekong Sub region and prevent its global spread.

Furthermore, Mbengue et al. (2015) study provided biochemical and cellular evidence that Artemisinin are potent inhibitors of *Plasmodium falciparum* phosphatidylinositol-3-kinase (PfPI3K), revealing an unexpected mechanism of action. In resistant clinical strains, increased PfPI3K was associated with the C580Y mutation in *P. falciparum* Kelch13 (PfKelch13), a primary marker of Artemisinin resistance. Polyubiquitination of PfPI3K and its binding to PfKelch13 were reduced by the PfKelch13 mutation, which limited proteolysis of PfPI3K and thus increased levels of the kinase, as well as its lipid product phosphatidylinositol-3-phosphate (PI3P). PI3P levels were found to be predictive of artemisinin resistance in both clinical and engineered laboratory parasites as well as across non-isogenic strains.

Menard et al. (2016) identified 108 non synonymous K13 mutations, which showed marked geographic disparity in their frequency and distribution. In Asia, 36.5% of the K13 mutations were distributed within two areas, one in Cambodia, Vietnam, and Laos and the other in western Thailand, Myanmar, and China with no overlap. In Africa, a broad array of rare non synonymous mutations that were not associated with delayed parasite clearance were observed. No evidence of Artemisinin resistance was found outside Southeast Asia and China, where resistance-associated K13 mutations were confined. The common African A578S allele was not associated with clinical or in vitro resistance to Artemisinin, and many African mutations appear to be neutral.

In contrast, Juliana et al. (2018) found that frequent recrudescence of ACT-treated *P. falciparum* infections occurs with or without K13 mutations and emphasize the need for improved partner drugs to effectively eliminate the parasites that persist through the ACT component of combination therapy. Furthermore, Dong et al. (2018) in a study in Yunnan Province and Myanmar reported that the genetic diversity of k13 was higher in the *Plasmodium* isolates from Yunnan Province than those from Myanmar cases. The genetic differentiation index of the two populations was 0.0410, where the intra- and inter-group variations were 95.9% and 4.1%, respectively. The odds ratio of Artemisinin resistance phenotype and mutation at the locus 446 in k13 gene in Myanmar cases was 1.640, while the value was 1.840 based on the estimations of the mutations in the 12 loci.

Abubakar et al. (2020) in their study in Nigeria found that a fragment of the kelch13 gene encompassing the propeller domains was sequenced in 49 samples, alongside

samples of the susceptible strain pf_3D7. Low polymorphism was observed, suggesting a lack of selection on this gene. In addition, Scott et al. (2018) in a modelling study predicted that artemisinin-resistant strains may circulate up to 10 years longer in high compared to low *P. falciparum* prevalence areas before resistance is confirmed. Decreased time-to-resistance in low prevalence areas was explained by low genetic diversity and immunity, which resulted in increased probability of selection and spread of artemisinin-resistant strains.

Artemisinin resistance was estimated to be established by 2020 in areas of Africa with low (< 10*%) P. falciparum* prevalence, but not for 5 or 10 years later in moderate (10–25%) or high (> 25%) prevalence areas, respectively. Grigg et al. (2018) found that all 278 *P. falciparum* isolates evaluated were wild-type for Kelch13 markers. There is no suspected or confirmed evidence of endemic artemisinin-resistant *P. falciparum* in the pre-elimination setting in Sabah, Malaysia. Current guidelines recommending first-line treatment with ACT remain appropriate for uncomplicated malaria in Sabah, Malaysia.

Yobi et al. (2020) found that of the 717 successfully sequenced *P. falciparum* isolates, 710 (99.0%; 95% CI: 97.9% - 99.6) were wild type genotypes and 7 (1.0%; 95% CI: 0.4% - 2.1%) carried non-synonymous (NS) mutations in pfk13-propeller including 2 mutations (A578S and V534A) previously detected and 2 other (M472I and A569T) not yet detected in the DRC. Mutations associated with ACT resistance in Southeast Asia were not observed in DRC. Imwong et al. (2017) found that in 2014–15, a single long PfKelch13 C580Y haplotype (–50 to +31·5 kb) lineage, which emerged in western Cambodia in 2008, was detected in 65 of 88 isolates from northeastern Thailand, 86 of

111 isolates from southern Laos, and 14 of 14 isolates from western Cambodia, signifying a hard transnational selective sweep.

However, Zaw et al. (2017) reported that to date, 13 non-synonymous mutations of k13 gene have been observed to have slow parasite clearance. Worldwide mapping of k13 mutant alleles have shown mutants associated with Artemisinin resistance were confined to southeast Asia and China and did not invade to African countries.

2.3.4 Occurrence *P. falciparum* Kelch13 R561H mutants among malaria cases

Paloque et al. (2016) reported that artemisinin resistance is due to mutation of the PfK13 propeller domain and involves an unconventional mechanism based on a quiescence state leading to parasite recrudescence as soon as drug pressure is removed. The enhanced *P. falciparum* quiescence capacity of artemisinin-resistant parasites results from an increased ability to manage oxidative damage and an altered cell cycle gene regulation within a complex network involving the unfolded protein response, the PI3K/PI3P/AKT pathway, the PfPK4/eIF2α cascade and yet unidentified transcription factor(s), with minimal energetic requirements and fatty acid metabolism maintained in the mitochondrion and apicoplast.

In contrast, Intharabut et al. (2018) in their study found that early changes in parasitemia level (0–6 hours after admission) were determined mainly by modal stage of asexual parasite development, whereas the subsequent log-linear decline was determined mainly by kelch13 propeller mutations. Older circulating parasites on admission were associated with more-rapid parasite clearance, particularly in kelch13 mutant infections. The geometric mean parasite clearance half-life decreased by 11.6% (95% CI 3.4%–19.1%)

in kelch13 wild-type infections and by 30% (95% CI 17.8%–40.4%) in kelch13 mutant infections as the mean age of circulating parasites rose from 3 to 21 hours.

However, He et al. (2019) found that major mutations in the K13 propeller domain associated with artemisinin resistance in the Mekong region (C580Y, R539T and Y493H) were absent, but F446I and two previously undescribed mutations (V603E and V454I) were identified. Furthermore, Fairhurst et al. (2016) have established that artemisinin resistance manifests as slow parasite clearance in patients and increased survival of early ring-stage parasites in vitro; is caused by single nucleotide polymorphisms in the parasite's 'K13' gene; is associated with an upregulated "unfolded protein response" pathway that may antagonize the pro-oxidant activity of artemisinins; and selects for partner drug resistance that rapidly leads to ACT failures. Hott et al. (2015) found that drug effects on asexual stages was found to correlate with parasite clearance half-life in vivo as well as with mutations in the Kelch domain gene associated with resistance (Pf3D7_1343700).

WWARN et al. (2019) found that both the pfk13 genotypes and the PC1/2 were available from 3250 (95%) patients (n = 3012 from Asia (93%), n = 238 from Africa (7%)). In addition, mutant allele E252Q located in the *P. falciparum* region of pfk13 was associated with 1.5-fold (95%CI 1.4–1.6) longer PC1/2. None of the isolates from four countries in Africa showed a significant difference between the PC1/2 of parasites with or without pfk13 propeller region mutations.

Ocan et al. (2018) also reported that artemisinin resistance has not been widely reported among parasites in Africa and other malaria-endemic countries outside Southeast Asia.

However, several studies on artemisinin resistance have reported different K13-gene polymorphisms from the validated mutations found in Southeast Asia. In contrast Ocan et al. (2019) found that the aggregate prevalence of single pfKelch13 gene was 27.6% (3694/14,827) (95% CI 22.9%, 32.3%). Sub-group analynucleotide polymorphisms (SNPs) in sis showed that aggregate prevalence of non-synonymous SNPs in pfkelch13 gene was higher, 45.4% (95% CI 35.4%, 55.3%) in Southeast Asia as opposed to 7.6% (95% CI 5.6%, 9.5%) in the African region.

A total of 165 independent K13 mutations were identified across malaria-affected regions globally. A total of 16 non-validated K13 mutations were associated with increased ACT parasite clearance half-life (t1/2 > 5 h). The majority, 45.5% (75/165), of the mutations were reported in single *P. falciparum* parasite infections. Of the 165 k13-mutations, over half were reported as new alleles. Diversity of mutations in pfkelch13 gene is highest in African region compared to SEA. Li et al. (2019) found that of the 319 *P. falciparum* samples, 12 samples had the k13-propeller mutation, and 11 point mutations were detected; 5 were non-synonymous mutations (T474I, A481T, A578S, V603E, G665S) and were not associated with artemisinin resistance. Two cases were treated with artemisinin for 8 days with a 960-mg dose to completely clear the asexual parasite, but they did not have a mutation in the K13 gene.

In related studies Straimer et al. (2015) found that isolates from Cambodia, where resistance first emerged, survival rates decreased from 13 to 49% to 0.3 to 2.4% after the removal of K13 mutations. Conversely, survival rates in wild-type parasites increased from ≤0.6% to 2 to 29% after the insertion of K13 mutations. These mutations conferred

elevated resistance to recent Cambodian isolates compared with that of reference lines, suggesting a contemporary contribution of additional genetic factors.

Stanley et al. (2020) suggested that the decrease in hemoglobin-derived heme reduces artemisinin activation, which is sufficient to enable parasite survival in the early ring stage of infection.

Li et al. (2016) found that the sequences of K13-propeller and PfATPase6 were obtained from 90.74% (98/108) and 91.45% (139/ 152) samples, respectively. The 2.04% (2/98) cases had non-synonymous K13-propeller A578S mutation but none found the mutations associated with ACT resistance in Southeast Asia. Mayengue et al., 2018 showed a very limited polymorphism in the K13-propeller gene in isolates from the Republic of Congo and K13 polymorphisms associate with ACT resistance are not present in that country.

Bornington et al. (2017) found that the proportion of K13 mutations in patients was 41.7%, and only 5 alleles were detected: C580Y, I205T, M476I, R561H, and F446I. Of these, F446I was the most common, and was associated with a longer parasite clearance half-life (median) 4.1 (min–max 2.3–6.7) hours compared to 2.5 (min–max 1.6–8.7) in wild type (p=0.01). The prevalence of K13 mutant parasites was much lower than the proportion of K13 mutants detected 200 km south of Myanmar in a much less remote setting where the prevalence of K13 mutants was 84% with 15 distinct alleles in 2013 of which C580Y predominated.

Furthermore, Oboh et al. (2018) found that none of the different validated and candidate AA mutations of PfK13 gene conferring resistance to ACT could be detected in *P.*

falciparum sampled in the two south western states of Nigeria. Therefore, based on the monomorphism at the PfK13 gene and non-association of mutations of this gene with mutations in three other drug-resistant genes in malaria parasite *P. falciparum*, it can be proposed that malaria public health is not under immediate threat in south western Nigeria concerning ACT resistance.

However, Woodrow et al. (2017) reviewed clinical efficacy data from the region (2000–2015) that provides strong evidence that the loss of first-line ACTs in western Cambodia, first Artesunate-Mefloquine and then DHA-Piperaquine, can be attributed primarily to K13 mutated parasites. The ring-stage activity of artemisinin is therefore critical for the sustained efficacy of ACTs; once it is lost, rapid selection of partner drug resistance and ACT failure are inevitable consequences. Although multiple K13 mutations have arisen independently, successful multidrug-resistant parasite genotypes are taking over and threaten to spread to India and Africa.

2.3.5 Risk factors of *P. falciparum* malaria ACT treatment failure cases and the PfKelch13 R561H mutation among malaria cases

2.3.5.1 Availability of antimalarial drugs

Khuluza et al. (2017) in Malawi found that availability of the antimalarial artemether/lumefantrine and Sulfadoxine/pyrimethamine, which are provided with financial support from international donors, was high in public and Christian Health Association of Malawi (CHAM) facilities (93% and 100%, respectively). Nevertheless, for 10 of the 12 investigated medicines the cost for one course of treatment exceeded the daily wage of a low-paid government worker in Malawi and therefore had to be

considered as unaffordable for a major part of the population. In contrast, Manirakiza et al. (2010) found that availability of drugs was recorded in all drugs wholesalers (n= 3), all pharmacies in health facilities (n= 14), private drugstores (n= 15), and in 60 non-official drug shops randomly chosen in the city. Artemisinin derivatives used in monotherapy or in combination were commonly sold. In health care facilities, 93% of the uncomplicated malaria cases were treated in the absence of any laboratory confirmation and the officially recommended treatment, amodiaquine-sulfadoxine/pyrimethamine, was seldom prescribed. Thus, the national guidelines for the treatment of uncomplicated malaria were not followed by health professionals in Bangui. In addition, Kindermans et al. (2007) reported that identifying and obtaining data from the private sector was difficult.

The quality of information on drug supply and distribution in countries must be improved. Current adult treatment prices for ACTs range US$ 1–1.8. Taking the upper range for both volumes and costs, the highest number of adult treatments consumed for Africa was estimated at 314.5 million, corresponding to an overall maximum annual need for financing ACT procurement of US$ 566.1 million. In reality, both the number of cases treated and the cost of treatment are likely to be lower (projections for the lowest consumption estimate with the least expensive ACT would require US $ 113 million per annum (Kindermans, 2007).

In another study Angira et al. (2010) revealed that all health facilities were using general-purpose trucks to transport antimalarial drugs and did not have functional wall thermometers and that eighty-seven per cent (87%) of health care providers did not check storage conditions of drugs upon reception. Ninety-seven per cent (97%) of the health care providers used physical examination for clinical diagnoses that is subject to

errors that may lead to irrational drug use. Thirteen per cent (13%) of health care providers had no idea that antimalarials suspensions can undergo fermentation when not properly stored. Forty percent (40%) of the selected health facilities had current recommended antimalarial treatment drugs in stock. The use of such vehicles can affect the potency of the drugs, as they do not have the necessary equipment to control adverse temperatures and this may contribute to loss of potency. Some health facilities did not have the current recommended antimalarial drugs in stock implying that patients attending treatment in these facilities could have been treated with less effective drugs or they could have been sent to purchase them yet they are expensive and not easily available.

However, Smith et al. (2011) found that Quinine is the most frequently stocked anti-malarial (61% of retailers). More medicine retailers stocked sulphadoxine-pyramethamine (SP; 57%) than ACT (44%). Eleven percent of retailers stocked AMFm subsidized artemether-lumefantrine (AL). The mean price of any brand of AL, the recommended first-line drug in Kenya, was $2.7 USD. In addition, Okoro et al. (2018) found that overall, 95.8% of the total prescriptions contained ACTs, out of which 80.8% were Artemether/Lumefantrine.

2.3.5.2 Misuse of ACTs

Nwokolo et al. (2017) in their study reported misuse of ACTs following overtreatment of malaria based on clinical diagnosis occurs when suspected cases of malaria are not prior confirmed with a test. Non-testing before sales of malaria medicines by pharmacies will perpetuate ACT misuse with the patients not benefiting due to poor treatment outcomes, waste of medicines and financial loss from out-of-pocket payment for

unneeded medicines. Inappropriate use of Artemisinin derivatives can reduce *P. falciparum* susceptibility (Shahinas, 2020).

2.3.5.3 Prevention of Artemisinin resistance

Dondorp et al. (2017); Adelaide et al. (2017) proposed that for containment of artemisinin resistance, an accelerated elimination strategy will be needed. This includes high-quality implementation of conventional malaria control measures: early case management with quality artemisinin combination therapies (avoiding artesunate monotherapies) and single gametocytocidal low dose of primaquine, vector control and surveillance. Village health workers (VHWs) play a key role in the provision of community-based services which have to reach even the most remote populations. Additional, more aggressive, approaches will be important to accelerate malaria elimination, which could include mass drug administrations, potentially in combination with ivermectin and vaccination, mass screening and treatment with novel diagnostics, reactive case detection, and other measures. In addition, Tun et al. (2017) have developed an open-access, user-friendly, web-based tool to analyses parasite clearance half-life data of *P. falciparum* infected patients after treatment with artemisinin derivatives, so that resistance to Artemisinin can be identified. The tool can be accessed at bit.ly/id_artemisinin_ resistance.

Nguyen et al. (2015) that use of Multiple First Line Treatment(MFT) was predicted to reduce the long-term number of treatment failures compared with strategies in which a single first-line ACT is recommended. This was robust to various epidemiological, pharmacological, and evolutionary features of malaria transmission. Inclusion of a single non-ACT therapy in an MFT strategy would have substantial benefits in reduction of

pressure on artemisinin resistance evolution, delaying its emergence and slowing its spread. However, Suresh et al. (2018) found that where elevation of a lipid, phosphatidylinositol-3- phosphate (PI3P) results in vesicle expansion that increases the engagement with the unfolded protein response (UPR). Vesicle expansion efficiently induces artemisinin resistance likely by promoting 'proteostasis' to mitigate artemisinin-induced proteopathy. Vesicular amplification engages the host red cell, suggesting that artemisinin resistant malaria may also persist by taking advantage of host niches and escaping the immune response.

2.3.5.4 Adherence to Artemisinin

Onyango et al. (2012) reported that adherence to ACT prescription remained low at 42.1% and 57.9% among individuals above 13 and less than 13 years, respectively. Stratification by demographic and socio-economic characteristics in relation to ACT adherence revealed that age ($P = 0.011$), education level ($P < 0.01$), ability to read ($P < 0.01$) and household (HH) monthly income ($P = 0.002$) significantly affected the level of ACT adherence. In addition, about 52.9% of the respondents reported that ACT was not always available at the source and that drug availability ($P = 0.020$) and distance to drug source ($P < 0.01$) significantly affected accessibility.

In contrast, Yakasai et al. (2015) found that the prevalence of ACT adherence in the public sector was significantly higher compared to retail sector (76% and 45%, resp., $P < 0.0001$). Factors found to be significant predictors of ACT adherence were years of education ≥ 7 (odds ratio (OR) (95% CI) = 1.63 (1.05–2.53)), higher income (2.0 (1.35–2.98)), fatty food (4.6 (2.49–8.50)), exact number of pills dispensed (4.09 (1.60–10.7)), and belief in traditional medication for malaria (0.09 (0.01–0.78)). To improve ACT

adherence, educational programs to increase awareness and understanding of ACT dosing regimen are interventions urgently needed. Patients and caregivers should be provided with an adequate explanation at the time of prescribing and/or dispensing ACT.

Chatio et al. (2015) study revealed educated ones had a good knowledge on ACTs and preferred artemether lumefantrine in treating their malaria. The reason was that the drug was good and it had minimal or no side effects. Perceived cure of illness after the initial dose greatly affected adherence. Other factors such as forgetfulness and lack of information also influenced patient adherence to ACTs use. Furthermore, Banek et al. (2014) in a meta-analysis found that most studies only reported whether medication regimens were completed, but did not assess how the treatment was taken by the patient (i.e. timing, frequency and dose). However, Amponsah et al. (2015) in their study found that adherence levels to the ACTs were 57.3%. Patient related factors that affected adherence to ACTs were patients' knowledge on the dosage (P = 0.007; V = 0.457), efficacy (P = 0.009; V = 0.377), and side effects (P = 0.000; V = 0.403) of the ACTs used for the management of malaria, patients' awareness of the consequences of not completing the doses of antimalarial dispensed (P = 0.001; V = 0.309), and patients' belief that "natural remedies are safer than medicines" and "prescribers place too much trust in medicines." There is the need for pharmacy staff to stress on these variables when counseling patients on antimalarials as these affect adherence levels.

Takahash et al. (2018) found that of the 54 patients examined, 51 (94.4%) were adherent to the AL regimen. The other three patients stopped medication because they felt better, even though the importance of completing the regimen was explained to all patients when it was prescribed. The healthcare workers perceived the major reasons for poor

adherence to be illiteracy and poor understanding of medication instructions by patients. In practice, 27.6% of the healthcare workers did not regularly explain the importance of completing the regimen to patients, and 32.2% did not often or always confirm the patients' understanding of medication instructions.

2.3.6 Artemisinin content of ACT drugs obtained from community vendors

Estimates suggest that up to 60% of antimalarial circulating in sub-Saharan African countries could be fake and may contribute to over 116,000 deaths annually in the region alone (WHO,2019). Falsified and poor-quality medicines significantly contribute to the development of drug resistance. It is well known that resistance to one of the most efficient and commonly used component drugs for malaria treatment, artemisinin, first appeared in a part of the world (Greater Mekong Sub region) where between 38 and 90% of the eponymous medicines on the market were either substandard or falsified. In addition, Kaur et al. (2016) reported that the teams purchased over 10,000 samples, from six malaria endemic countries: Equatorial Guinea (Bioko Island), Cambodia, Ghana, Nigeria, Rwanda and Tanzania. Laboratory analyses of these samples showed that falsified anti-malarias (<8 %) were found in just two of the countries, whilst substandard artemisinin-based combinations were present in all six countries and, artemisinin-based monotherapy tablets are still available in some places despite the fact that the WHO has urged regulatory authorities in malaria-endemic countries to take measures to halt the production and marketing of these oral monotherapies since 2007. However, Kakolwa et al. (2018) reported that all the tested ACT in mainland Tanzania were highly efficacious and none of validated K13 mutants associated with Artemisinin resistance was observed.

Thomas et al. (2018) revealed that one reason for malaria's continued virulence in the developing world is ineffective medicine. In fact, in some poor African countries, many malaria drugs are actually expired, substandard or fake. Globally, some 200,000 preventable deaths occur each year due to anti-malarial drugs that do not work. Substandard and counterfeit medicines may be responsible for up to 116,000 malaria deaths annually in sub-Saharan Africa alone, according to recent World Health Organization estimates. Fraudulent pharmaceuticals are on the rise. Reports of counterfeit or falsified anti-malarias rose 90 percent between 2005 and 2010, according to a 2014 article in the Malaria Journal. Furthermore, in 2012, a research team from the U.S. National Institutes of Health found that about one-third of anti-malarial medicines distributed in Southeast Asia and sub-Saharan Africa were of poor quality. A few years prior, fully 44 percent of anti-malarial supplies in Senegal had failed quality control tests. That's because counterfeiting pharmaceutical drugs is profitable business for manufacturers. This illegal activity is most common in places with little government oversight and limited access to safe, affordable and high-quality medicines. Various reports have found that many fake medicines originate in India, followed by China, Hong Kong and Turkey.

In addition, Battersby et al. (2003) found that many unregistered drugs are readily available, and poor storage conditions are likely to reduce the efficacy of drugs even if they were of good quality at the time of manufacture. For many people the cost of even the cheapest antimalarial is an issue and purchase of part doses is common. The knowledge of the staff in pharmacies is poor and in shops woefully inadequate. Nonetheless most people use shops and private pharmacies as their source for drugs. Karunamoorthi et al. (2014) also found that a sizable number of patients are unable to

afford Artemisinin Combination Therapy first-line treatment. Consequently, patients tend to procure cheaper anti-malarials, which may be fake or substandard. Fragile drug regulations, ineffective law-enforcement agencies and corruption further burden ailing healthcare facilities. Substandard/fake anti-malarials can cause (a) economic sabotage; (b) therapeutic failure; (c) increased risk of the emergence and spread of resistant strains of *Plasmodium falciparum* and *Plasmodium vivax*; (d) an undermining of trust/confidence in healthcare stakeholders/systems; and, (e) serious side effects or death.

Thomas et al. (2012) reported that counterfeit antimalarials (mainly artemisinin derivatives) is a crucial health problem in developing countries, particularly in Africa. The illegal production, sale and distribution of fake drugs is a huge market evaluated to several billions of dollars and represents more than 50% of the pharmaceutical market in several African countries. Fake drugs have led to a very great number of deaths from untreated malaria or fatality provoked by toxic ingredients. These fake medicines increase the risk of artemisinin resistance developed by the use of sub therapeutic dosages of antimalarials.

Palafox et al. (2014) found that regulatory compliance varied across countries, particularly with respect to licensing. The proportion of wholesalers possessing any up-to-date licence from national regulators was lowest in Benin and Nigeria, where vendors in traditional markets are important antimalarial supply sources.

CHAPTER 3 METHODOLOGY

3.1 Introduction

This chapter outlines the research design, study setting, study population, sampling criteria and methods, data collection methods, data analysis plan and ethical considerations.

3.2 Research design

A 1:3 unmatched case-control study (Kim et al., 2016) was conducted out to solicit more information on status of artemisinin-based combination Therapy (ACT) resistance-

conferring *Plasmodium falciparum* malaria Kelch-13 R561H polymorphism in Mutasa District, Zimbabwe. **Cases** are defined as patients returning with malaria symptoms within 1 month of treatment. **Controls are** defined as patients that do not return with malaria symptoms within 1 month of treatment. The research design was chosen for its strength in determining association between outcome and factor variables. In addition, Artemisinin treatment failure proportions are still low in Africa, therefore case-control study with efficient identification of treatment failures and controls and helps show causality information between variables within the limited time period available to conduct the study. Finally, this study design allows for comparison of factors/reasons, why given the same circumstances some of the ACT treatments are successful whilst others are failing.

3.3 Study setting

The study was conducted in Mutasa District at 3 randomly chosen clinics with suspected ACT treatment failure records which are St Peters, Chinaka and Chisuko. Mutasa has a hot, temperate climate and high rainfall pattern ideal for vector breeding and malaria transmission. Perennial streams and gravity fed irrigation channels span the low-lying *Honde Valley* part of the district, creating a number of breeding sites for malaria vectors, mosquitoes. Mutasa District is located in the eastern part of Zimbabwe and shares its border with Mozambique. Malaria transmission is highly seasonal, peaking during the rainy season between November and April, and the main vector is *Anopheles funestus*. The District is home to large-scale tea estates, as well as small scale subsistence farmers.

3.4 Study population

3.4.1. Inclusion criteria: Participants of all age groups who tested RDT or slide microscope positive, received ACT treatment and present again at health facility with malaria symptoms within a month

3.4.2 Exclusion criteria: Participants of all age groups who tested RDT or slide microscopy positive, received treatment and present again at health facility with malaria symptoms after 1 month.

Participants with *P. falciparum* RDT positive test result were enrolled in Mutasa District.

3.5 Sample size calculation

Sample size was calculated using Epi Info 7 stat calculator function with the following parameters: study Power= 80%, Ratio of controls to cases= 3 (Kim et al., 2016), Percent of controls exposed=2.7 (Uwimana et al., 2020), Percent of cases with exposure= 6.7%. Therefore, a sample size of 934 was obtained with 234 cases and 700 controls

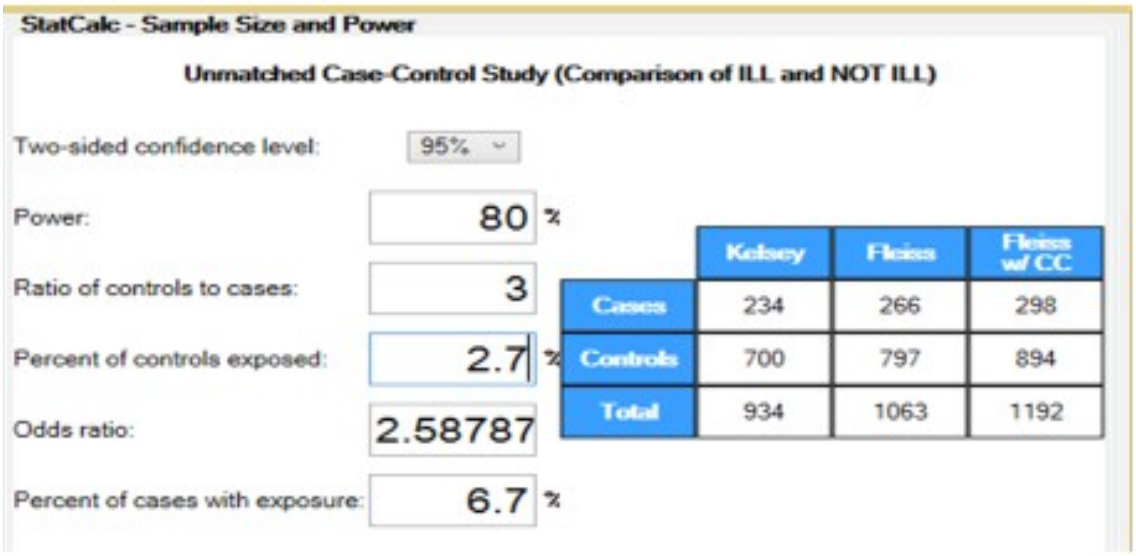


Fig 3.1 Sample size determination (Epi info)

3.6 Sampling procedure

Participants presenting at the selected clinics within a month after initial ACT treatment, RDT positive were purposely selected to participate. Participants 16 years and older were asked for consent, while parents or guardian were asked to assent for their children. Blood were collected onto a filter paper from a finger using a needle prick. Dried Blood Spot samples were transported to Africa University Laboratories.

3.6.1 Laboratory Methods

3.6.1.1. Artemether RDT assay

3.6.1.1.1 Extraction

The following Standard Operating Procedure was used for the extraction Artemether active ingredients. Crush the drug tablet in a folded piece of paper to a fine powder. Then transfer the powder to >95% alcohol to produce a content of 2mg/ml based on the

labelled content of commercial drug and mix well. Discard the paper. Do not reuse the paper for the next test to avoid contamination. For the artemether soft capsules and artemether injection, directly dissolve the drug to >95% alcohol to produce a content of 2mg/ml and shake well.

3.6.1.1.2 Dilution

Using the pipette, transfer 25ul of the solution prepared in step 1, into 25ml water in a cup. The diluted concentration is approximately 2ug/ml of Artemether in theory (the indicator range is 500-1000 ng/mL for artemether).

3.6.1.1.3 RDT analysis

Put a drop of sample into sample well and put drops enough into the diluent well. Allow the sample to move by capillary for about 15 minutes. Observe colour development on the control and test lines. If there is one line developing on control line only, then the test is regarded as negative. If colour develops at both test and control lines, the test is regarded as positive(fake). Record the results after 15 minutes.

3.6.1.2 Molecular analysis of *Plasmodium falciparum* Kelch 13 R561 H genotyping

3.6.1.2.1 Deoxyribonucleic Acid (DNA) Extraction for Nested PCR

Chelex DNA extractions: The lab scientist will begin by making the following solutions:1x PBS with 0.1% saponin weight/volume and distilled water with 2% Chelex weight/volume. The procedure as follows:

Cut 1 dried blood spot into the appropriate 1.5 ml tube that is labeled with the same participant number as the filter paper. Add 1mL of the 1x PBS/saponin solution. Incubate the tubes at room temperature for 10 minutes. Centrifuge the samples for 2

minutes at 14,000 rpm. Discard the supernatant (liquid). Add 1mL of 1x PBS to each tube. Centrifuge the samples for 2 minutes at 14,000rpm. Discard the supernatant (liquid). Add 150 µl of the dH_20/Chelex solution to each tube. Add 50µl dH_20 to each tube. Close the tubes and pierce a hole in each cap with a needle. Incubate the samples at 100˚C for 8 minutes. Centrifuge the samples at 14,000 rpm for 1 minute. Transfer the supernatant to the second set of labeled tubes taking care that the sample from one tube goes into the tube with the same label. The samples must be stored at -20˚C.

3.6.1.2.2 Plasmodium falciparum Kelch 13 R561 H genotyping

The primers for typing the *P. falciparum* Kelch13 R561H codon were designed at the Africa University molecular biology using the laser gene software and primer sequences are as follows: K13mFW: 5'-GGGGGATATGATGGCTCTTC-3'**;** K13mRV1: 5'GCTATTAAAACGGAGTGACCAA-3'**;** K13mRV2: 5'TTAAAACGGAGTGACCAAATCTG-3'**.**

3.6.1.2.3 K13R561H codon Polymerase Chain Reaction (PCR) COMPOSITION:

For Semi-Nest 1 (K13MFW/K13MRV1) and Semi-Nest 2 (K13MFW/K13MRV2) we used, in 25 µl reactions: 2.0 template; 0.25 µM primers; 1.5 mM magnesium chloride; 200 µM dNTP's; 1X PCR buffer; 1.0U Taq DNA polymerase.

3.6.1.2.4 K13 R561H CODON AMPLIFICATION

Annealing cycles for both Semi-Nest 1 and Semi-Nest 2 reactions were as follows: 1 cycle of denaturation at 94˚C, 2 minutes, 25 cycles of: 94˚C denaturation for 45 seconds, 51˚C annealing for 45 seconds, 65˚C extension for 1 minute and 1 cycle of final extension at 65˚C for 2 minutes

- **Gel electrophoresis:** We load 5µl of PCR product per lane on 2% agarose gel and run at 100V for 60 minutes. Semi-Nest II product is **538bp**. (Primary product is 542bp)

3.6.1.2.5 Restriction Fragment Length Polymorphisms(RFLP) K13 Codon R561H

All samples amplified with 538bp band were subjected to NdeI digestion overnight @ 37 degrees Celsius. Complete digestion yields 320 base pair and 200bp bands (wild type) while undigested samples remain with 538base pair (mutant type)

- **Nde I Digest Composition** in 30µl reaction: (5.0 µl amplicon DNA; 1X NE Buffer, 1U Nde I) overnight digestion at 37°C

  Load 13µl of digest per lane of 1.5-2% agarose gel and run at 100V. Use the same volume of loading dye (approx. 1µl) as usual.

3.7 Data collection tools

A structured questionnaire was administered to all consenting participants to capture the factors contributing to malaria treatment failures in Mutasa District. A separate structured questionnaire was administered to community vendors and private outlets selling drugs in communities. Laboratory forms and records were used to record genotyping results and ACT rapid test findings.

3.8 Research variables

3.8.1 Dependent variables

In this study, malaria ACT treatment failure is the dependent variable. This was ascertained through measuring variables such number of malaria cases returning with

malaria symptoms within a month after full course of ACT administration and genotyping Pf Kelch 13 R561H mutants.

3.8.2 Independent variables

The demographic and socio-economic characteristics of the malaria cases, knowledge, attitudes, adherence to ACT drugs, misuse of ACT drugs and health systems factors were the independent variables. This included the age, education level, and occupation, religion, health facility storage of ACTs, and ACT drug availability both in public, private sector and informal vendors.

3.9 Pretesting of data collection tools

Pretesting was done at Tsvingwe Clinic, Premier Clinic, Old Mutare hospital for feasibility, reliability and validity. The questionnaire was evaluated for test-retest reliability using 4 cases and 4 controls. The samples were randomly selected at the study sites and there are excluded from the larger study sample. Face and construct validity will be enhanced through subjecting the questionnaire to review by experts in public health and aligning the research instrument to the conceptual framework for spread of Artemisinin resistance in populations. Furthermore, back translating the local language questionnaire to English to ensure reliability.

3.10 Data analysis

In this study, Epi info version 7 was used for capturing, cleaning and tabularisation of all the data collected using questionnaires. Univariate analysis was employed to describe distribution of artemisinin resistance. In order to determine the association between

quality of ACT drugs and treatment failure, a bivariate analysis was conducted. Multivariate logistic regression was used to test for association of Kelch-13 mutants, and factors of malaria treatment failure as well as to generate Odds ratios. Thematic analysis will be used to analyze qualitative data. Data will be presented in graphs and tables.

3.11 Ethical considerations

Ethical approval for the study was sought from Africa University Research Ethics committee (AUREC) and permission to conduct research in Mutasa was sought at District Medical Officer (DMO) Mutasa, Manicaland. A written consent was obtained from all participants prior to each interview session. All the participants were assured that they can withdraw from the study whenever they want with no disadvantage to their care. Privacy and confidentiality was maintained throughout the study. Measures to ensure confidentiality such as telling clients that no information sharing to other people and privacy were maintained, no names were used on the questionnaires but were coded using numbers. Furthermore, data were kept in a safe and lockable cupboard.

CHAPTER 4 DATA PRESENTATION, ANALYSIS AND INTERPRETATION

4.1 Introduction

This chapter presents, describes and explains the findings from the data collected and analyzed using EPI info 7. A univariate, bivariate and multivariate analysis was done for the variables. Tables, graphs and pie charts were used to show the data focusing on socio-demographic characteristics, reasons for not getting tested before taking ACTs drugs, reasons for buying ACTs drugs from vendors, proportion of participant prefer buying ACT drugs from private pharmacy/ vendors and Average cost of ACT drugs sold in Mutasa. This chapter also presents the interpretation and discussion of the key results of the study in light of the study conceptual framework. The study hypothesised that socio-demographic characteristics of suspected malaria treatment failure cases, their knowledge and attitudes on ACT drug adherence, quality of ACT drugs and mutations in the *Plasmodium falciparum* Kelch13R561H genes factors are associated with suspected malaria treatment failure Mutasa District. These assumptions were confirmed in this study as presented in the discussion of key results in this section.

4.2 Socio-demographic characteristics

The majority of participants were males constituting 57.1%(n=47) while females were 43%(n=35). The mean age for both cases and controls was 41.9 years and the median was 42 years. Interquartile range for the age was Q1=17 and Q3=80. The majority of cases and controls stayed in St Peters 49(62%) while 38%(n=33) stayed in Chinaka and Chisuko clinics. Both cases and controls had equal proportions with regards to

employment, as most participants reported farming as occupation. Mutasa District is empowering people to do farming as a source of income rather than just for subsistence. Majority of the cases (75%) reported that their nearest health facility is not within 1 km, while 25% cases reported that their nearest health facility is indeed within 1 km. All the socio-demographic characteristics were not associated with suspected treatment failure as shown in Table 4.1 This could be attributed to the low number of malaria cases reported and enrolled in the study as the 2020/2021 malaria season reported very low malaria cases.

Table 4.1 Socio-demographic characteristics

variable	**category**	**case**	**control**	**or (95% CI)**	**p-value**
Sex	Male	15	32	0.5	0.142
	Female	17	18	(0.2-1.2)	
Age group	<25	5	8	0.9	0.922
	≥25	27	42	(0.3-3.3)	
Clinic	St Peters	20	29	1.2	0.730
	Other	12	21	(0.5-3.0)	
Occupation	Unemployed	16	30	0.7	0.442
	Employed (formal/informal)	16	20	(0.3-1.6)	
Nearest health facility within 1km	No	24	29	2.2	0.131
	Yes	8	21	(0.8-5.8)	

4.3 Source of ACT

The study findings show that the majority of respondents) get the ACT drugs from Hospitals, get their ACT from public clinics and get from private pharmacy/ vendors as shown in Fig 4.1 below.

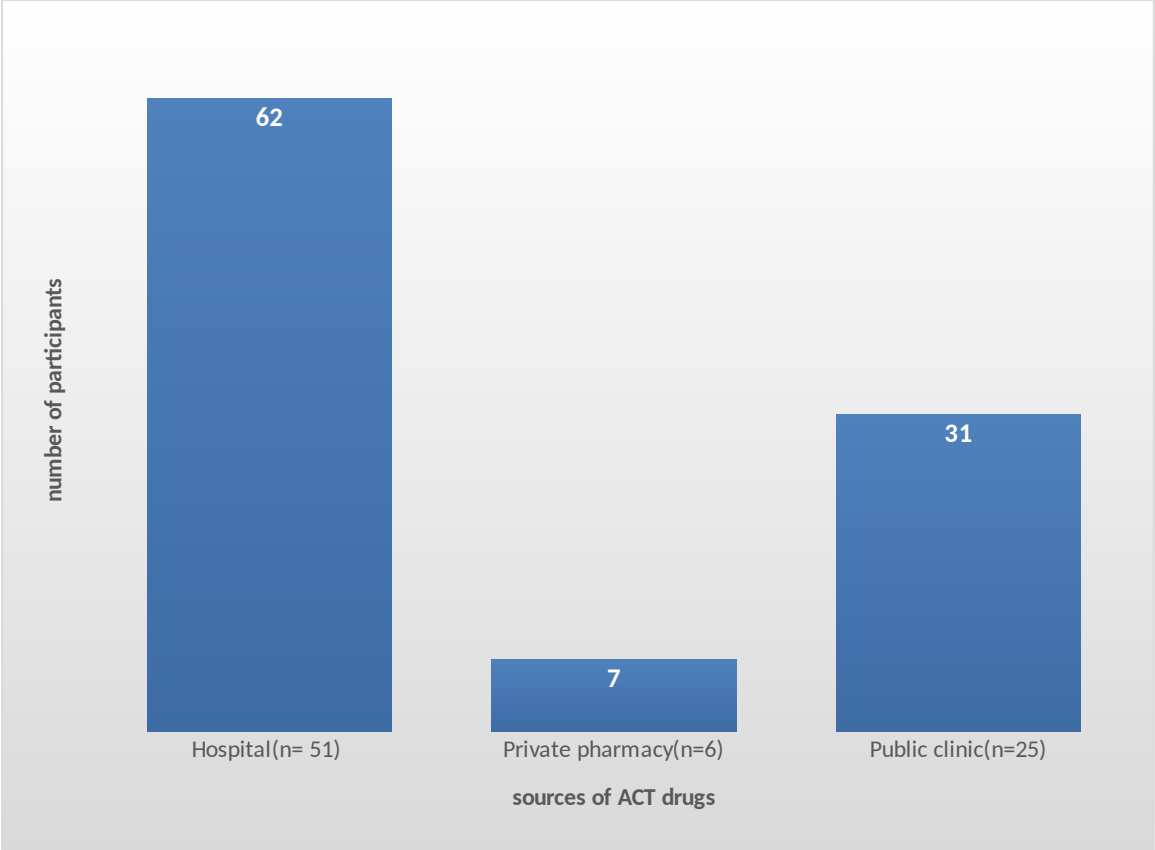


Fig 4.1: Sources of ACT in Mutasa District

4.4 Reasons for not getting tested for malaria before getting ACT

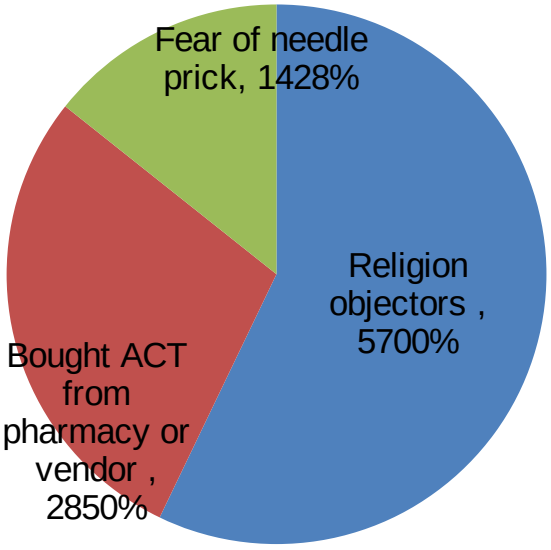


Fig 4.2 Reasons for not getting tested for malaria before getting ACT

The study findings show that the majority 57.2% of respondents don't get tested for malaria before getting ACT drugs, 29.04% of respondents did not get tested because they bought ACT from a pharmacy/ vendor while 14% don't get tested due to fear of the needle prick as shown in Fig 4.2.

4.5 Reasons for ever buying ACT from vendors

The study findings show that majority of respondents reported that out of stocks of ACT drugs at the clinic is the main reason for buying ACT drugs from community vendors while 38.1% of respondents indicated that tested malaria negative at the clinic was a reason for buying drugs from vendors.as shown in Fig 4.3

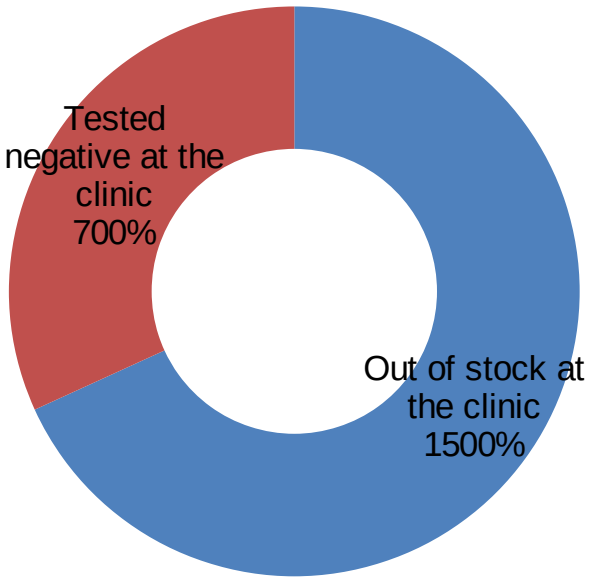


Fig 4.3 Reasons for buying ACT from Vendors

4.6 Other antimalarial medication and methods being used to treat malaria in Mutasa communities

The study findings show that majority of respondents 36.0% use Paracetamol, *pararogo,* salt and sugar solution as other antimalarials drugs.34.2% respondents reported that there is no other antimalarial drug used except coartem. Only a very small proportion(n=2) of respondents mentioned using Quinine as other antimalarial drug they use. The study finding show that the Mutasa community use various other methods to treat malaria such as traditional herbs (9.1%), amoxylin antibiotics, brufen and pain killers, chilli plant pepper, drinking lots of water and steaming ginger, *kubvuvata kupinda mubafu (steaming hot water inside blanket)* and using mosquito nets.

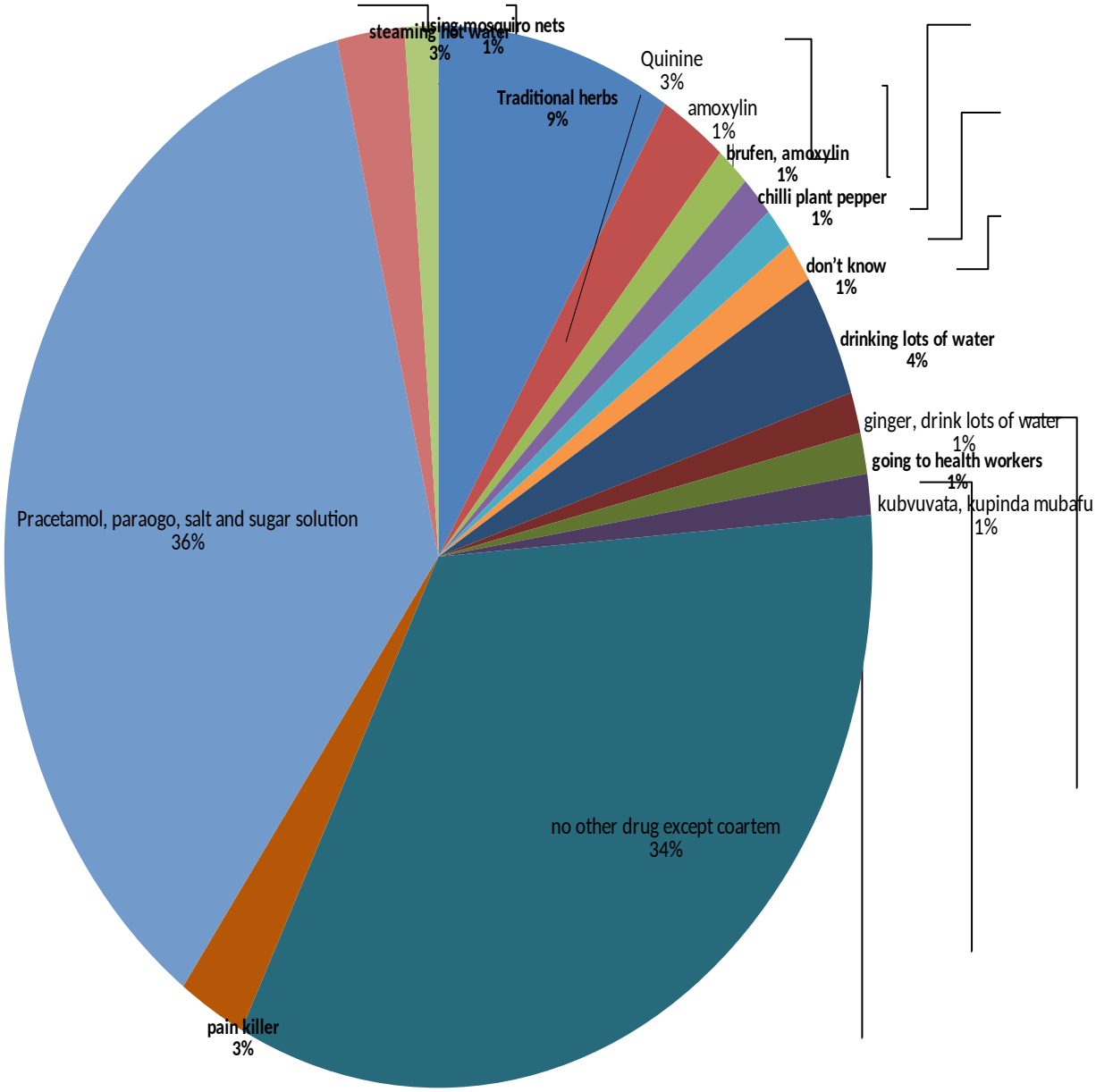


Fig 4.4 Other anti-malarial drugs used

4.7 Participants behaviour on buying ACTs from Vendors

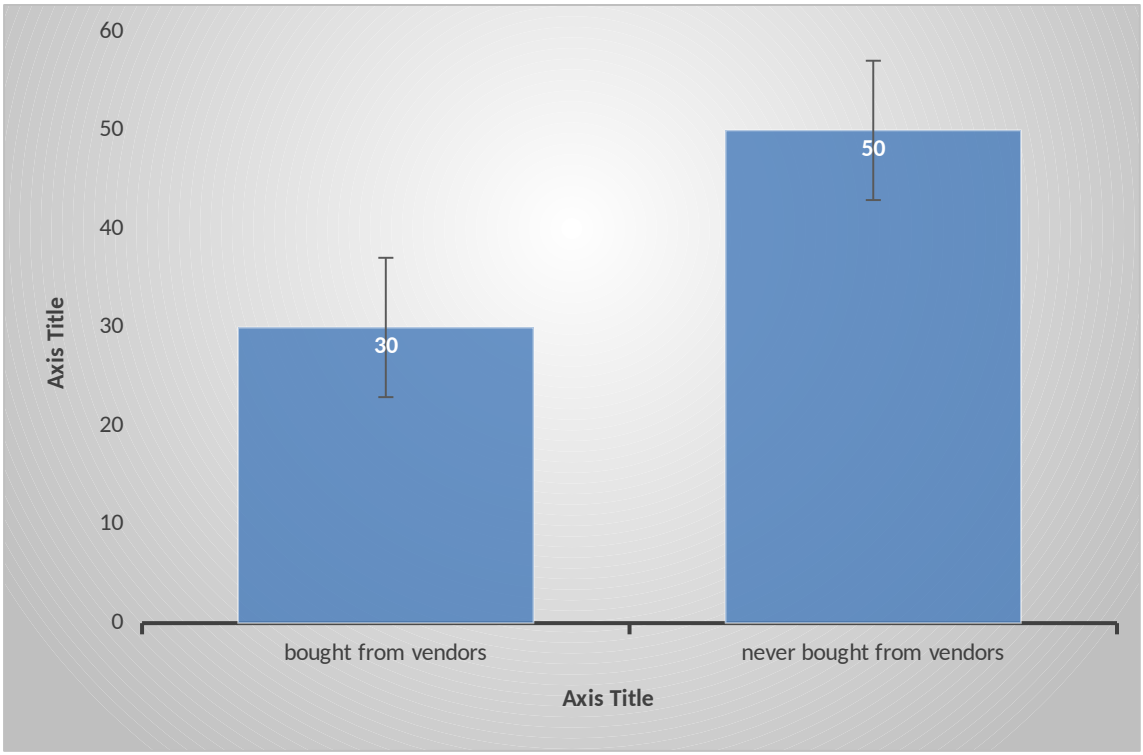


Fig 4.5 Participants behavior on buying ACTs from Vendors

The study findings show that majority of respondents(n=50) never bought ACT drugs from vendors while other respondents(n=30) reported buying ACT drugs from vendors as shown in fig 4.5 above.

4.8 Average cost of ACT drugs sold in Mutasa

The study findings show that most respondents said ACT drugs are not sold and are given for free. However, some respondents said course of ACT cost USD1(n=10) and prices range from 1 USD to 10USD as shown in Fig 4.6 below.

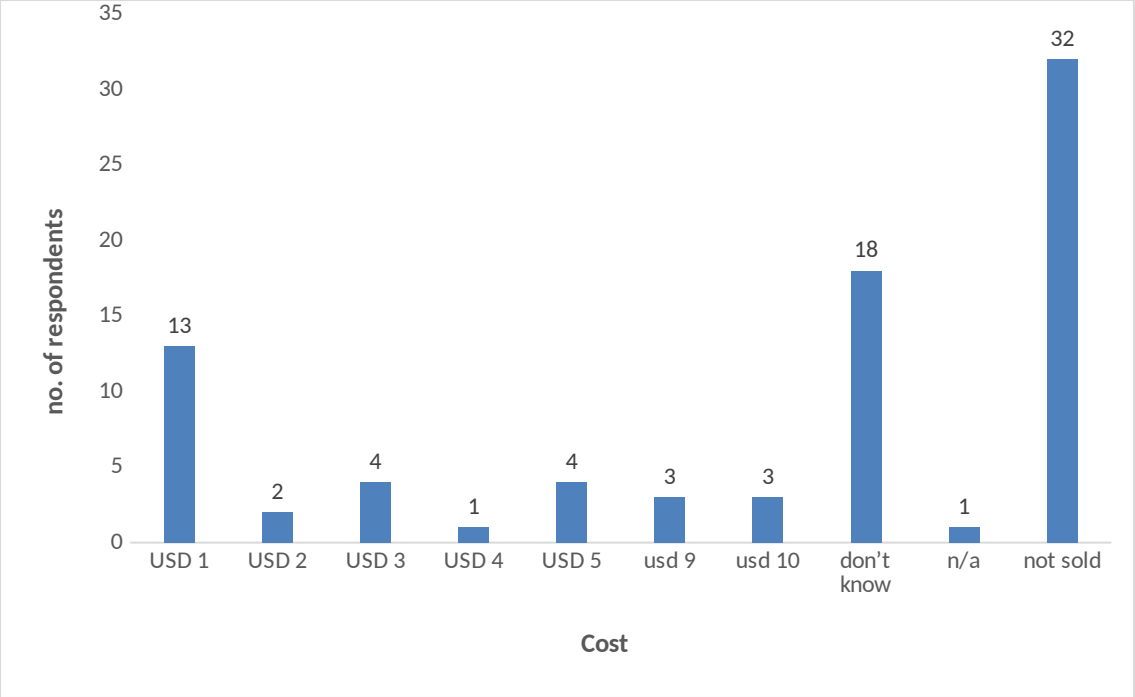


Fig 4.6 Average cost of ACT drugs sold in Mutasa

4.9 Influencing factors for buying ACTs from vendors

The study findings show that respondents have various factors that drive them to buying ACT drugs from vendors. The factors comprise of not have been tested for malaria, failing to get drugs from hospital due to out of stock, failing to get ACT drugs from clinic, tested negative for malaria and decided to buy and lastly, some respondents said health facilities are selling to vendors and they will resell to communities as shown in fig 4.7.

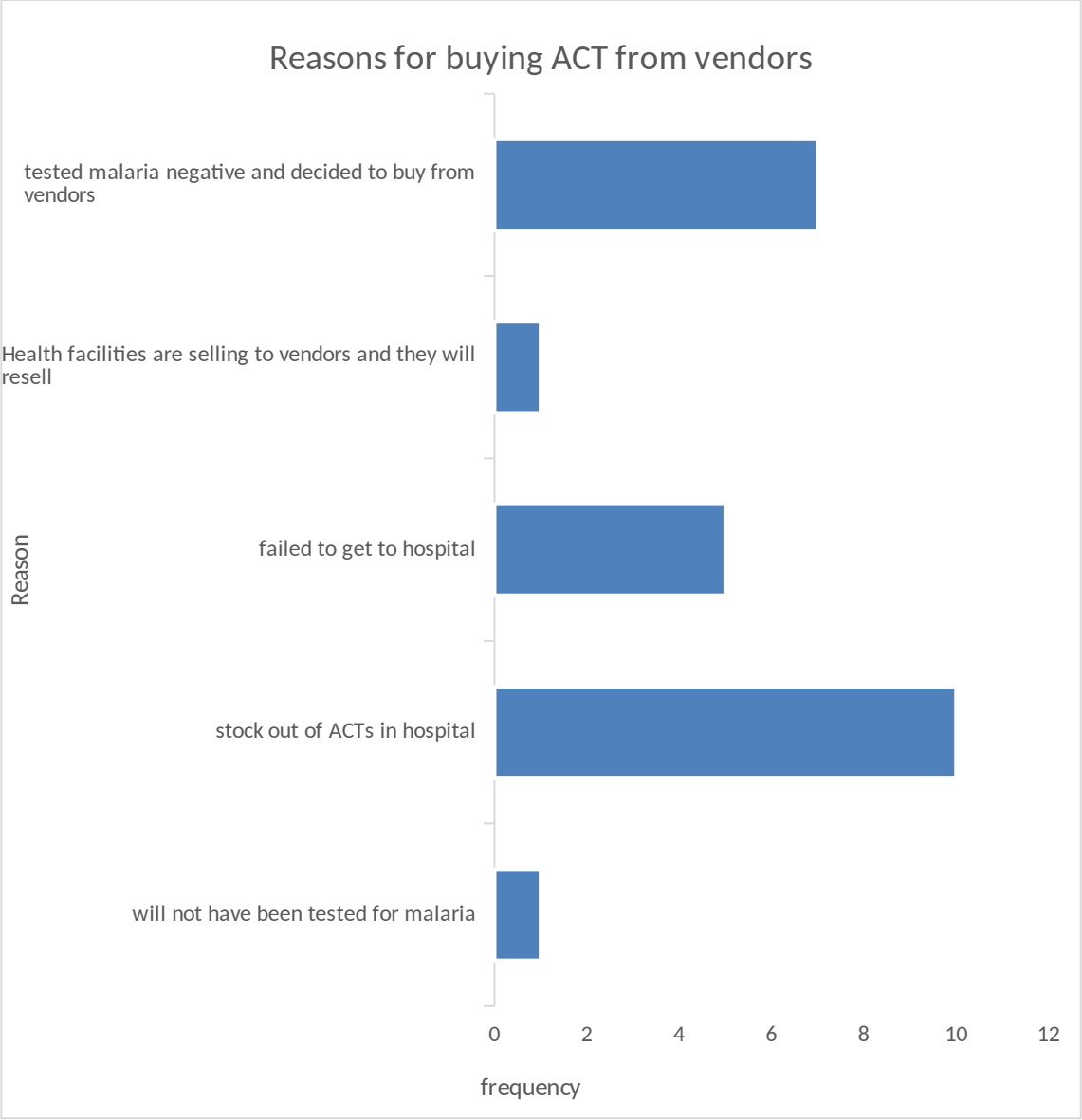


Fig 4.7 Influencing factors for buying ACTs from vendors

4.10 Suspected malaria treatment failure risk factors

A Bivariate analysis of the risk factors for suspected malaria treatment failure was done. The risk factors analysed comprised ACT readily available(p-value=0.523), test for malaria before getting ACT(p-value=0.403), patient received health education regarding ACT adherence(p-value=0.536), Completed ACT course(p-value=0.411), there were

community campaigns on ACT use(p-value=0.240), experienced side effects after taking ACT(p-value=0.418), received counselling on ACT use(p-value=0.614) and ever bought ACT from vendors(p-value=0.912). All these risk factors were not associated with suspected malaria treatment failure.

Table.4.2 Suspected malaria treatment failure risk factors

**frequency of cell contents is less than 5 and the Chi-squared test of association may not be valid*

Factor	Response	case	control	OR (95% CI)	p-value
ACT is readily available at the clinic					
	No	5	5	1.7	0.523
	Yes	27	45	(0.4-6.3)	
Patient test for malaria before getting ACT					
	No	2	6	0.5	0.403
	Yes	30	44	(0.1-2.6)	
Patient received health education on ACT use					
	No	3	7	0.6	0.536
	Yes	29	43	(0.2-2.7)	
Completed ACT course					
	No	1	4	0.4	0.411
	Yes	31	46	0.04-3.5)	
There were community campaigns on ACT use					
	No	4	12	0.4	0.240
	Yes	29	37	(0.1-1.5)	
Experienced side effects after taking ACT					
	No	25	42	0.6	0.418
	Yes	8	7	(0.2-1.9)	
Received counselling on ACT use					
	No	13	21	0.8	0.614
	Yes	20	28	(0.3-2.0)	

Factor	Response	case	control	OR (95% CI)	p-value
Have ever bought ACT from vendors					
	No	20	31	1.0	0.913
	Yes	12	18	(0.4-2.4)	
K13R561H codon SNP PCR Genotyping					
	Positive	26	1*	212	0.001
	Negative	6	49	(24.2-1859)	

4.11 Laboratory Results (Genotyping of *P. falciparum* St Peters 2021 DBS *P. falciparum* Kelch 13R561H codon gel results

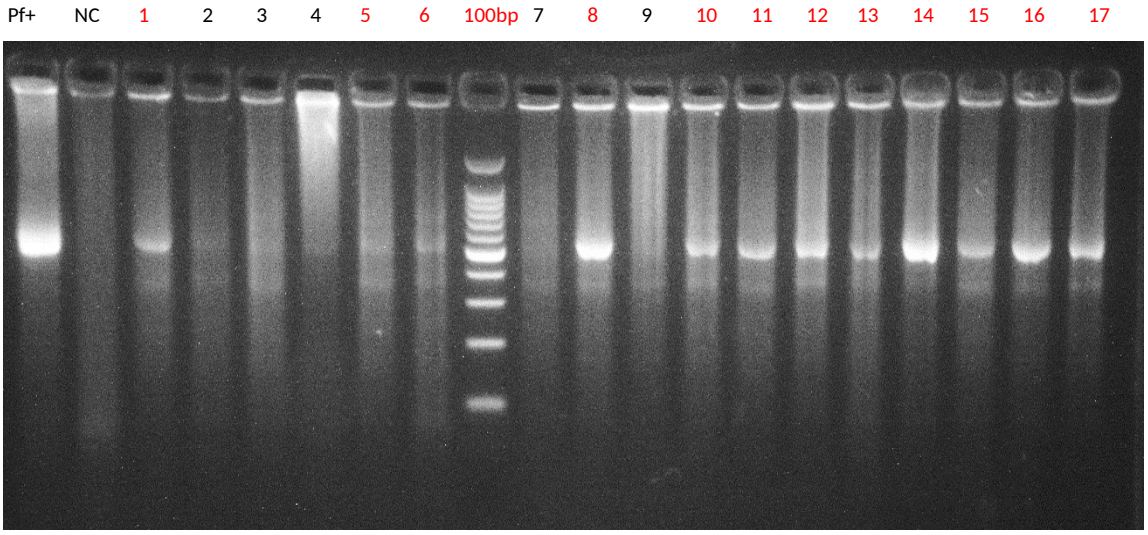


Fig 4.8 shows the gel results for *P. falciparum* Kelch 13 R561H codon. Lane Pf+ (positive control), lane NC (Negative Control), lane 1-17(Samples), Lane 100bp(100 base pair ladder), Lane 1,5,6,8,10-17(K13 R561H positive samples amplified with 538bp ladder)

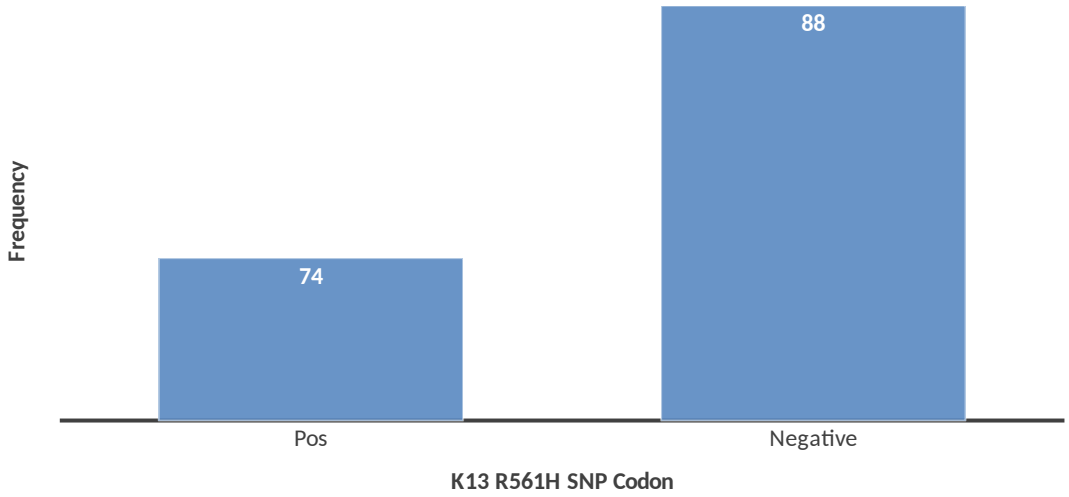


Fig 4.9 Graph of K13 R561H SNP Codon Prevalence in Mutasa and lab repository samples collected in 2003 before ACT were introduced in Zimbabwe.

4.12 K13 R561H codon NdeI RFLP (Nde I Restriction Endonuclease Digestion at 37 Degrees Celsius overnight

K13R561H RFLP	CASES	CONTROL
K13 R561H Wild type	20	1
K13R561H Mutant type	6	0
N/A	6	49

Table 4.3: Showing K13 R561H Codon which were subjected to Nde1 RFLP

Table 4.3 shows that of the k13 R561H codon samples which were Nde I Restriction Fragment Length Polymorphisms endonuclease digested, only 6 samples from cases were found to be K13R561H mutant type. 20 samples from cases and 1 sample from controls were found to be k13R561H wild type.

Pf+ NC 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

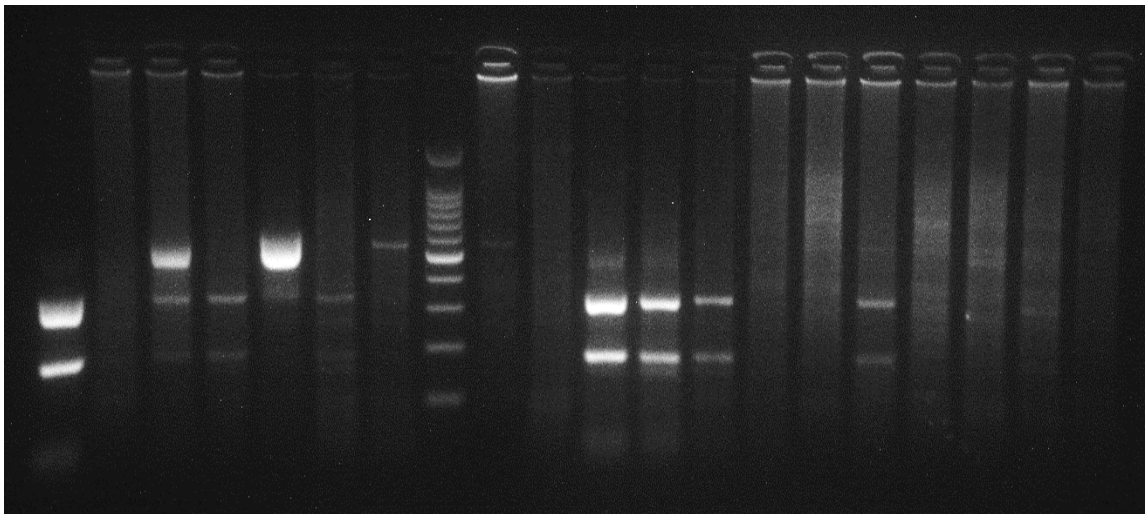


Fig 4.10: Gel electrophoresis of RFLP products. Lane Pf+ (positive control wild type, complete digestion), lane 1,3(mutant type, undigested products, partial digestion), lane 7(mutant type, faint undigested product), lane 9-10(mutant type, Incomplete digestion).

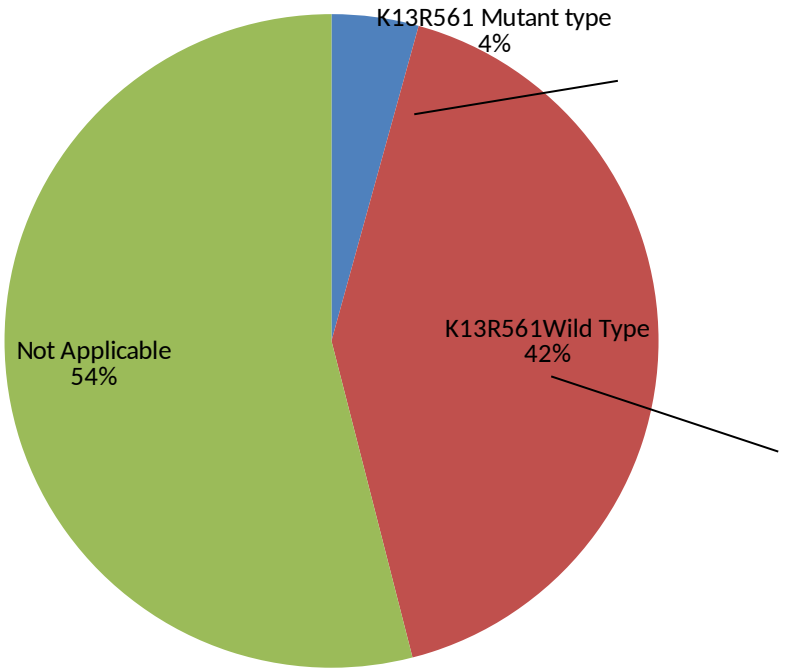


Figure 4.11 Proportion of K13R561H Nde I RFLP

Fig 4.11 shows that k13R561H mutant type constitute 4.3% of the analyzed samples, 42.0% wild type and 54.1% of samples that did not meet criteria for RFLP i.e no k13R561H amplification.

4.13 Comparison of K13 R561H Codon Restriction Endonuclease digest RFLP between 2003 & 2021 samples

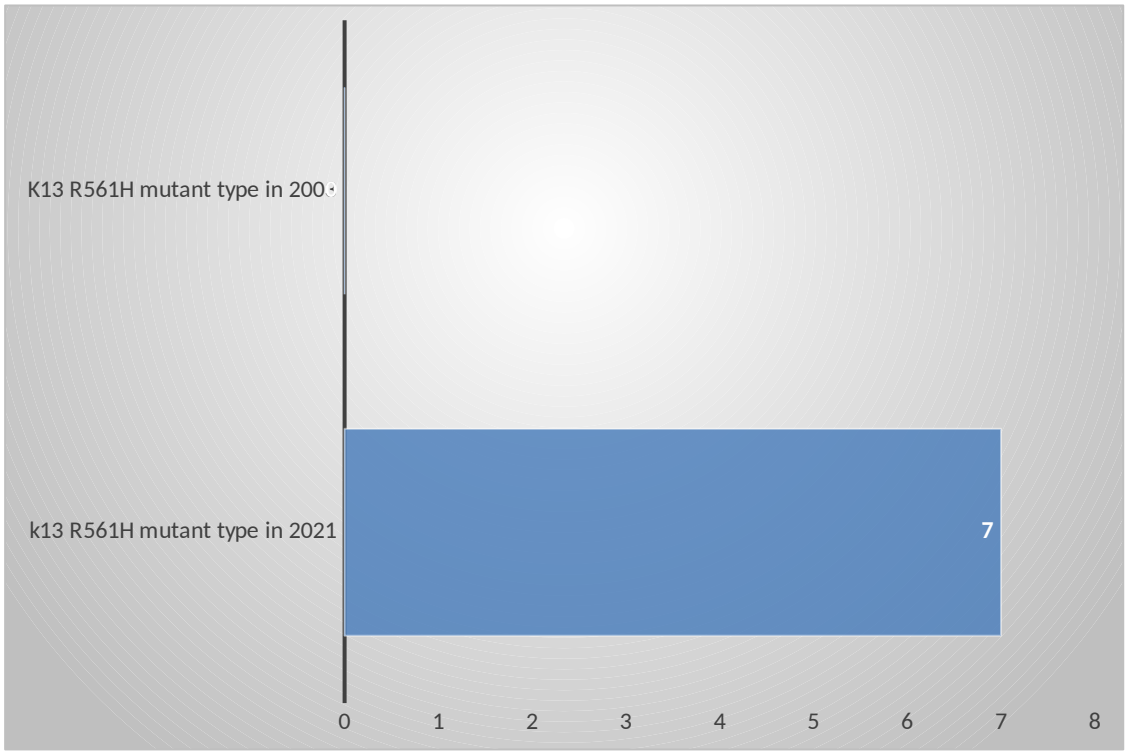


Fig 4.12 Comparison of K13 R561 H mutant type between 2003 & 2021

Fig 4.12 shows that in 2003, there was no k13 R 561H mutant type while in 2021 there was 7 samples that show k13R561H mutant type.

4.14. Artemisinin Drugs Quality Composition Determination using the Dipstick Assay

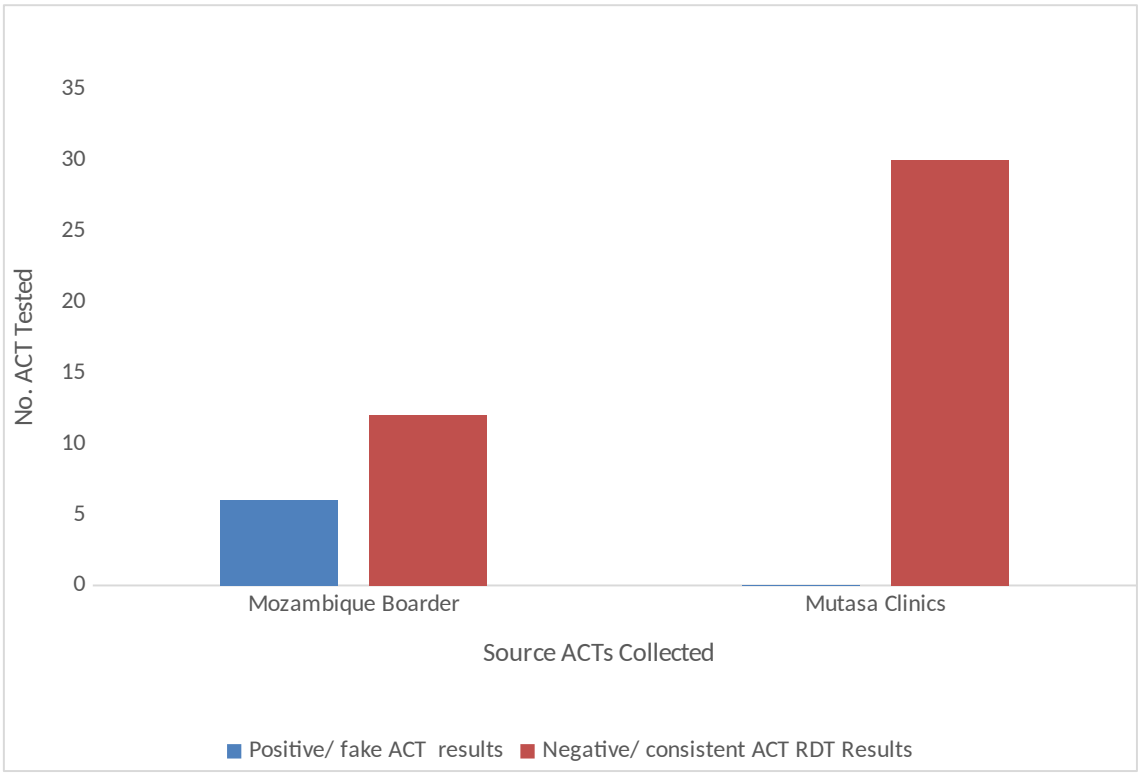


Fig 4.13 ACT drug quality composition results

Fig 4.13 shows that 5 ACT drugs collected from Mozambique boarder were positive by RDT dipstick and were fake ACTs. However, these were not labelled. All the ACT bought from Mozambique side were negative, consistent with the ACT composition. All ACT drugs obtained from Zimbabwe side in Mutasa had labels and were RDT dipstick negative confirming that the ACT drug quality was consistent with acceptable standards

4.15 VHW Mode of transport used to carry ACT drugs from the health facility in Mutasa

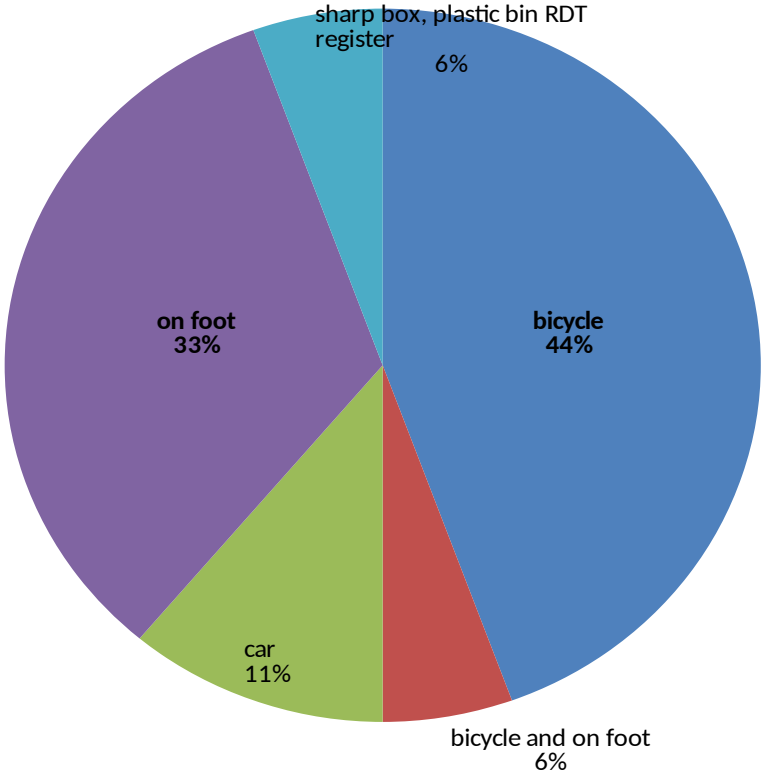


Fig 4.14 VHW Mode of transport used to carry ACT drugs from HC

Fig 4.13 shows that majority (83.0%) of village health workers' mode of transport used to carry ACT drugs from Health Centres is bicycle and on foot. Only 11.4% use cars without thermometers fitted to check temperatures.

CHAPTER 5 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 Introduction

The chapter discusses study findings in relation to what other researchers found, draw conclusions and recommendations

5.2 Discussion

The study findings show that the majority of respondents 62.2%(n=51) get the ACT drugs from Hospitals, 31.3%(n=25) get their ACT from public clinics and 7.0%(n=6) get from private pharmacy/ vendors. The results are consistent with findings of Khuluza et al. (2017) in Malawi who found that availability of the antimalarial artemether/lumefantrine and Sulfadoxine/pyrimethamine, was high in public and Christian Health Association of Malawi (CHAM) facilities (93.4% and 100%, respectively). However, Smith et al. (2011) found that quinine was the most frequently stocked anti-malarial (61% of retailers). More medicine retailers stocked sulphadoxine-pyramethamine (SP; 57%) than ACT (44%).

Eleven percent of retailers stocked AMFm subsidized artemether-lumefantrine (AL). The study findings show that most respondents said ACT drugs are not sold and are

given for free. However, some respondents said course of ACT drug costs USD1(n=10) and prices range from 1 USD to 10USD in Mutasa District. The findings are consistent with other researchers, Khuluza et al. (2017) who reported that for 10 of the 12 investigated medicines the cost for one course of treatment exceeded the daily wage of a low-paid government worker in Malawi and therefore had to be considered as unaffordable for a major part of the population. In contrast, Kindermans et al. (2007) found that adult treatment prices for ACTs range US$ 1–1.8. In reality, both the number of cases treated and the cost of treatment are likely to be lower (projections for the lowest consumption estimate with the least expensive ACT would require US $ 113 million per annum). Battersby et al. (2003) reported that for many people the cost of even the cheapest antimalarial is an issue and purchase of part doses is common. The knowledge of the staff in pharmacies is poor and in shops woefully inadequate. Nonetheless most people use shops and private pharmacies as their source for drugs. Karunamoorthi et al. (2014) also found that a sizable number of patients are unable to afford ACTs first-line treatment.

The study findings show that respondents have various factors that drive them to buying ACT drugs from vendors. The factors comprise of not have been tested for malaria, failing to get drugs from hospital due to out of stock, failing to get ACT drugs from clinic, tested negative for malaria and decided to buy from vendors and lastly, some respondents said health facilities are selling to vendors and they will resell to communities. Findings were consistent with other researchers, Nwokolo et al. (2017) in their study reported that overtreatment of malaria based on clinical diagnosis occurs when suspected cases of malaria are not prior confirmed with a test. Non-testing before

sales of malaria medicines by pharmacies will perpetuate ACT misuse with the patients not benefiting due to poor treatment outcomes, waste of medicines and financial loss from out-of-pocket payment for unneeded medicines. Inappropriate use of artemisinin derivatives can reduce *P. falciparum* susceptibility (Shahinas et al., 2020).

A univariate analysis of the risk factors for suspected malaria treatment failure was done.

The risk factors analysed comprised ACT readily available(p-value=0.523), test for malaria before getting ACT(p-value=0.403), patient received health education regarding ACT adherence(p-value=0.536), Completed ACT course(p-value=0.411), community campaigns on ACT use(p-value=0.240), experienced side effects after taking ACT(p-value=0.418), received counselling on ACT use(p-value=0.614) and ever bought ACT from vendors(p-value=0.912). All these risk factors were not associated with suspected malaria treatment failure possibly due to the low sample size achieved in the current. Regarding adherence, Onyango et al., 2012 reported that adherence to ACT prescription remained low at 42.1% and 57.9% among individuals above 13 and less than 13 years, respectively.

In addition, about 52.9% of the respondents reported that ACT was not always available at the source and that drug availability ($P = 0.020$) and distance to drug source ($P < 0.01$) significantly affected accessibility. In contrast, Yakasai et al. (2015) found that the prevalence of ACT adherence in the public sector was significantly higher compared to retail sector (76% and 45%, resp., $P < 0.0001$). To improve ACT adherence, educational

programs to increase awareness and understanding of ACT dosing regimen are interventions urgently needed. Patients and caregivers should be provided with an adequate explanation at the time of prescribing and/or dispensing ACT.

Furthermore, Chatio et al. (2015) study revealed educated ones had a good knowledge on ACTs and preferred artemether lumefantrine in treating their malaria. The reason was that the drug was good and it had minimal or no side effects. Other factors such as forgetfulness and lack of information also influenced patient adherence to ACTs use. There is need for pharmacy staff to stress on these variables when counseling patients on antimalarials as these affect adherence levels.

The study findings show that K13R561H mutant type constitute 4% of the analysed samples, 42% wild type in Mutasa District. The presence of the K13 R561H mutant type shows that the suspected malaria treatment failure was attributed to the mutations in the K13 genes. The findings were consistent with other researches, Dondorp et al. (2009) who reported that recrudescence confirmed by means of polymerase chain-reaction(PCR) assay occurred in 6 of 20 patients (30%) receiving artesunate monotherapy and 1 of 20 (5%) receiving artesunate–mefloquine therapy in Pailin, as compared with 2 of 20 (10%) and 1 of 20 (5%), respectively, in Wang Pha (P = 0.31). Again, Ajay et al. (2013) in Nigeria reported three cases of early treatment failure due to possible artemisinin-based combination therapy-resistant *Plasmodium falciparum* malaria.

Ashley et al. (2014) also reported that slowly clearing infections (parasite clearance half-life >5 hours), strongly associated with single point mutations in the "propeller" region of the *P. falciparum* Kelch protein gene on chromosome 13 (Kelch13), were detected throughout mainland Southeast Asia from southern Vietnam to central Myanmar. In contrast, Patel et al. (2017) reported that no mutation was found in the K-13 propeller gene, while only one sample showed a mutant genotype for the PfATPase6 gene. The parasites remain sensitive to artemisinin derivatives. However, Laurent et al, 2018 found that nineteen non synonymous K13 mutations were present in the pre-ACT samples (N = 64) compared with 22 in the post-ACT samples (N = 251). In addition, another study by Ariey et al. (2014) found that mutant K13-propeller alleles cluster in Cambodian provinces where resistance is prevalent, and the increasing frequency of a dominant mutant K13-propeller allele correlates with the recent spread of resistance in western Cambodia. Strong correlations between the presence of a mutant allele, in vitro parasite survival rates and in vivo parasite clearance rates indicate that K13-propeller mutations are important determinants of artemisinin resistance. K13propeller polymorphism constitutes a useful molecular marker for large-scale surveillance efforts to contain artemisinin resistance in the Greater Mekong Sub region and prevent its global spread. Furthermore, Mbengue et al. (2015) study provided biochemical and cellular evidence that artemisinin are potent inhibitors of *Plasmodium falciparum* phosphatidylinositol-3-kinase (PfPI3K), revealing an unexpected mechanism of action. In resistant clinical strains, increased PfPI3K was associated with the C580Y mutation in *P. falciparum* Kelch13 (PfKelch13), a primary marker of artemisinin resistance.

In contrast, Juliana et al. (2018) found that frequent recrudescence of ACT-treated *P. falciparum* infections occurs with or without K13 mutations and emphasize the need for improved partner drugs to effectively eliminate the parasites that persist through the ACT component of combination therapy. Abubakar et al. (2020) in their study in Nigeria found that a fragment of the kelch13 gene encompassing the propeller domains was sequenced in 49 samples, alongside samples of the susceptible strain pf_3D7. Low polymorphism was observed, suggesting a lack of selection on this gene. In addition, Scott et al. (2018) in a modelling study predicted that artemisinin-resistant strains may circulate up to 10 years longer in high compared to low *P. falciparum* prevalence areas before resistance is confirmed. Decreased time-to-resistance in low prevalence areas was explained by low genetic diversity and immunity, which resulted in increased probability of selection and spread of artemisinin-resistant strains. Artemisinin resistance was estimated to be established by 2020 in areas of Africa with low (< 10*%) P. falciparum* prevalence, but not for 5 or 10 years later in moderate (10–25%) or high (> 25%) prevalence areas, respectively.

Yet another researcher Grigg et al. (2018) found that all 278 *P. falciparum* isolates evaluated were wild-type for Kelch13 markers. There was no suspected or confirmed evidence of endemic artemisinin-resistant *P. falciparum* in the pre-elimination setting in Sabah, Malaysia. Current guidelines recommending first-line treatment with ACT remained appropriate for uncomplicated malaria in Sabah, Malaysia. Also Yobi et al. (2020) found that of the 717 successfully sequenced *P. falciparum* isolates, 710 (99.0%; 95% CI: 97.9% - 99.6) were wild type genotypes and 7 (1.0%; 95% CI: 0.4% - 2.1%) carried non-synonymous (NS) mutations in pfk13-propeller including 2 mutations

(A578S and V534A) previously detected and 2 other (M472I and A569T) not yet detected in the DRC. Mutations associated with ACT resistance in Southeast Asia were not observed in DRC.

However, Imwong et al. (2017) found that in 2014–15, a single long PfKelch13 C580Y haplotype (–50 to +31·5 kb) lineage, which emerged in western Cambodia in 2008, was detected in 65 of 88 isolates from northeastern Thailand, 86 of 111 isolates from southern Laos, and 14 of 14 isolates from western Cambodia, signifying a hard transnational selective sweep. However, Zaw et al. (2017) reported that to date, 13 non-synonymous mutations of K13 gene have been observed to have slow parasite clearance. Worldwide mapping of K13 mutant alleles have shown mutants associated with artemisinin resistance were confined to southeast Asia and China and did not invade to African countries.

The study findings also show that five (5) ACT drugs collected from Zimbabwe-Mozambique boarder were positive by RDT dipstick and were fake ACTs. However, these were not labelled. All labelled ACT drugs bought from Mozambique side were negative, consistent with the ACT composition. All ACT drugs obtained from Zimbabwe side in Mutasa District had labels and were RDT dipstick negative confirming that the ACT drug quality was consistent with acceptable standards. Estimates suggest that up to 60% of antimalarial circulating in sub-Saharan African countries could be fake and may contribute to over 116,000 deaths annually in the region alone (WHO,2019).

Falsified and poor-quality medicines significantly contribute to the development of drug resistance. It is well known that resistance to one of the most efficient and commonly used component drugs for malaria treatment, artemisinin, first appeared in a part of the world (Greater Mekong Sub region) where between 38 and 90% of the eponymous medicines on the market were either substandard or falsified. In addition, Kaur et al. (2016) reported that the teams purchased over 10,000 samples, from six malaria endemic countries: Equatorial Guinea (Bioko Island), Cambodia, Ghana, Nigeria, Rwanda and Tanzania. Laboratory analyses of these samples showed that falsified anti-malarias (<8 %) were found in just two of the countries, whilst substandard artemisinin-based combinations were present in all six countries and, artemisinin-based monotherapy tablets are still available in some places despite the fact that the WHO has urged regulatory authorities in malaria-endemic countries to take measures to halt the production and marketing of these oral monotherapies since 2007.

In contrast, Kakolwa et al. (2018) reported that all the tested ACT in mainland Tanzania were highly efficacious and none of validated k13 mutants associated with Artemisinin resistance was observed. Thomas et al 2018 revealed that in some poor African countries, many malaria drugs are actually expired, substandard or fake. Globally, some 200,000 preventable deaths occur each year due to anti-malarial drugs that do not work. Substandard and counterfeit medicines may be responsible for up to 116,000 malaria deaths annually in sub-Saharan Africa alone, according to recent World Health Organization estimates. Fraudulent pharmaceuticals are on the rise. Reports of counterfeit or falsified anti-malarias rose 90 percent between 2005 and 2010, according to a 2014 article in the Malaria Journal. Furthermore, in 2012, a research team from the

U.S. National Institutes of Health found that about one-third of anti-malarial medicines distributed in Southeast Asia and sub-Saharan Africa were of poor quality. A few years prior, fully 44 percent of anti-malarial supplies in Senegal had failed quality control tests. That's because counterfeiting pharmaceutical drugs is profitable business for manufacturers. This illegal activity is most common in places with little government oversight and limited access to safe, affordable and high-quality medicines.

Thomas et al. (2012) reported that counterfeit antimalarials (mainly artemisinin derivatives) is a crucial health problem in developing countries, particularly in Africa. The illegal production, sale and distribution of fake drugs is a huge market evaluated to several billions of dollars and represents more than 50% of the pharmaceutical market in several African countries. Fake drugs have led to a very great number of deaths from untreated malaria or fatality provoked by toxic ingredients. These fake medicines increase the risk of artemisinin resistance developed by the use of sub therapeutic dosages of antimalarials. The current study also found that majority (83%) of village health workers' and community vendors' mode of transport used to carry ACT drugs from Health Centres is bicycle and on foot while only 11% use cars without fitted thermometers inside to check temperatures.

Findings are consistence with that of Battersby et al. (2003) who found that many unregistered drugs are readily available, and poor storage conditions are likely to reduce the efficacy of drugs even if they were of good quality at the time of manufacture. Consequently, patients tend to procure cheaper anti-malarials, which may be fake or substandard. Fragile drug regulations, ineffective law-enforcement agencies and corruption further burden ailing healthcare facilities. Substandard/fake anti-malarials can

cause (a) economic sabotage; (b) therapeutic failure; (c) increased risk of the emergence and spread of resistant strains of Plasmodium falciparum and Plasmodium vivax; (d) an undermining of trust/confidence in healthcare stakeholders/systems; and, (e) serious side effects or death**.** Palafox et al (2014) found that regulatory compliance varied across countries, particularly with respect to licensing. The proportion of wholesalers possessing any up-to-date licence from national regulators was lowest in Benin and Nigeria, where vendors in traditional markets are important antimalarial supply sources.

5.3 Conclusions

The suspected malaria treatment failure is attributed to the presence of the K13R561H mutations. Fake/ falsified antimalarial ACT drugs were observed in Mutasa District. None of the suspected malaria treated failure risk factors were associated with malaria treatment failures in Mutasa District. The newly lab developed assay k13R561H SNP assay can be used for monitoring ACT drug resistance and inform policy.

5.4 Limitations

Due to the low malaria burden in 2020/2021, the calculated sample size could not be achieved, hence most of the risk factors were not significant.

5.5 Recommendations

i. To conduct the study at a larger scale to map the artemisinin resistance in Zimbabwe using the newly developed k13 R561H tool.
ii. To scale up measures to reduce the spread of artemisinin resistance
iii. Increase educational campaigns on adherence and avoid sourcing ACT drugs from community vendors as there are fake drugs in circulation

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Appendix 1(a): Informed Consent Form for Participants

Good morning/ afternoon.

My name is Nobert Mudare a student at Africa University. I am carrying out a study on status of artemisinin resistance-conferring plasmodium falciparum malaria kelch-13 r561h polymorphism in Mutasa District, Zimbabwe. This form gives information about the study and will be used to document your willingness to participate in the study.

Purpose the study

The purpose of the study is to assess status of artemisinin resistance-conferring plasmodium falciparum malaria kelch-13 R561H polymorphism in Mutasa District, Zimbabwe. The study will provide updates on the global situation of Artemisinin drug resistance in Zimbabwe.

Benefits, Risk and Discomforts

There are no direct benefits for participating in this study. The information on therapeutic efficacy generated on Artemisinin Combination Thrwill be shared among malaria programme managers in order to provide the best possible advice to ministries of health. Risk of participating in this study are minimal. However, it may be possible that you may feel uncomfortable with some of the questions you may be asked. You may choose to skip them or to discontinue the interview process. This will not disadvantage your care at this health facility.

Duration and procedure

If you agree to be in the study, I would like to ask you some questions. I want to ask you about malaria medicines you have taken, what you know about malaria and what you do when you get sick. It will take about 15 minutes to ask you questions. I would also like

to take a small sample of blood from your finger. It will be no more than a few drops of blood on filter. Getting blood from you finger will take less than 5 minutes. The blood will be used to help understand the malaria parasite response to Artemisinin Combination Therapy treatment.

We will also ask if you will allow us to store your blood for future research. Your name will not be linked to the sample if they are used for future research. You can choose to be in the study but not have your sample used for future research if you want.

Should you agree to take part in this study you will undergo a face to face interview for 15 to 20 minutes while completing this form. You will also be asked to sanitize your hands with an alcohol based sanitizer or wash your hand with soap and running water, wear a face mask and maintain social distance in relation to COVID19 regulations throughout the interview process.

Confidentiality

Confidentiality will be maintained throughout the study. No name will appear on the questionnaire. Numbers will be assigned to each questionnaire for coding purposes. Any information obtained in connection with this study will not be linked with participants. The interviews will be conducted in private rooms. Participants who choose to withdraw from the study will be allowed to do so. Assurance will be given that their health care will not be compromised due to their decision. Your name will not be used in any report or publication that may arise from this study. After the study the

questionnaires will be stored in a lock and key cabinet and data stored in password protected computer files.

Additional costs

There will be no additional cost for you because of participating in this study save for the time spent while participating in this study.

Voluntary participation

Participation in this study is purely voluntary, if you decide to withdraw from this study your decision to withdraw will not affect your future health care services in any manner. If you decide to participate you are free to withdraw your consent and stop participating any time without any charges or penalties.

If you have any queries about this research study you can contact Dr E Mugomeri research supervisor on the following numbers 0776 167 964.

If you have concerns and complains regarding how the research is being conducted you can contact Africa University Research Committee on the following numbers +263 20 60024 or +263 20 60065 or email to aurec@africau.edu

Authorization

Before signing this consent form please ask any question on any aspect of this study that is unclear to you. You may take as much time to think about it. Signing this form indicates that l have read and understand the purpose of this study. My participation is

purely voluntary. Data collected will be used for the purpose of this study. By signing below l indicate my approval.

Signature of participants …….

Name of person obtaining this consent……

Date ……
DD/MM/YYYY

Statement by the researcher

I confirm that the participant was given an opportunity to ask question in regard to the study. All questions asked by the participant have been answered according to the best of my ability.

I confirm that the participant has not been coerced into giving consent and the consent has been given freely and voluntarily. A copy of this consent has been provided to the participant.

Name of Researcher: …….

Signature: ……

Date: …….
DD/MM/YYYY

Appendix 1 (b): Gwaro Retenderano

Gwaro retenderano rekupinda mutsvakurudzo

Musoro wetsvakurudzo: Status of Artemisinin Resistance-Conferring *Plasmodium falciparum* malaria Kelch-13 R561h Polymorphism in Mutasa District, Zimbabwe

Mutsvakurudzi: **Nobert Mudare**

MPH student Number: 160744 **Phone: 0779741234**

Zvamunofanira kuziva nezvetsvakurudzo ino

1. Muri kukumbirwa kupinda mutsvakurudzo.
2. Gwaro retenderano rino rinotsanangura tsvakurudzo iyi nezvamunotarisirwa kuita mutsvakurudzo.
3. Munokumbirwa kuti munyatsoverenga gwaro rino. Shandisai nguva yose yamungada pakuriverenga.
4. Munopinda mutsvakurudzo nokuda kwenyu uye munogona kusarudza kusapinda muzvirongwa zvetsvakurudzo. Kana muchinge mapinda mutsvakurudzo, munogona kubuda panguva chero ipi zvayo. Hapana zvamunoitwa nechikonzero chekuti munenge masarudza kubuda mutsvakurudzo.
5. Panguva yetsvakurudzo, tichakuzivisai kana tikawana ruzivo rutsva runogona kushandura pfungwa dzenyu maererano nekuti mungada kuramba muri mutsvakurudzo here kana kuti kwete.

Chinangwa chetsvakurudzo

Tinoda kudzidza nezvemishonga weArtemisinin Combination Therapy (ACTs) kuti ichiri kurapa marariya sezvinotarisirwa here. Tinoda kuziva kuti nemhaka yei vamwe vanhu vachirapwa chirwere chemarariya vachipora asi vamwe vasingapori nechirwere

chemarariya. Tinodawo kuziva kuti mukabatwa nemarariya munoita sei uye ndedzipi dzimwe nzira dzamunoshandisa kurapa marariya. Ruzivo rwatichaunganidza runofanira kubatsira mumabasa ekudzivirira chirwere chemarariya munharaunda menyu.

Chikonzero masarudzwa kupinda mutsvakurudzo

Masarudzwa kupinda mutsvakurudzo ino kuburikidza nekwamunogara. Munogara munzvimbo ichaitirwa tsvakurudzo uye imba yenyu yakasarudzwa pasina nzira chaiyo yaishandiswa kana kuti nechikonzero chekuti iri munzvimbo ine njodzi yekubatwa nechirwere chemarariya.

Zvichaitwa

Kana muchibvuma kupinda mutsvakurudzo ino, tichakubvunzai mibvunzo. Tinoda kukubvunzai mibvunzo maererano nezvemishonga yamakamboshandisa kurapa marariya, uye kuti munoita sei kana mukarwara nemarariya. Zvichatora nguva diki (ingangosvika maminitsi gumi nemashanu) kukubvunzai mibvunzo. Tichadawo kukutorai ropa shoma, madonhwe mashoma eropa kubva pamunwe wenyu. Tichashandisa ropa rose patsvakurudzo yekuda kunzwisisa utachiwana hunokonzera marariya kuti mishonga yemaArtemisinini Combination Therapy (ACTs) ichinesimba rinotarisirwa pakurapa marariya. Zviri kwamuri kutipa kana kusatipa ropa. Zita renyu harizotaridzwi paropa parinenge richizoshandiswa mune ramangwana. Munogona kusarudza kupinda mutsvakurudzo asi moramba kutipa ropa.

Zvamunowana

Hapana mubhadharo vamuchawana kana mapinda mutsvakurudzo iyi.

Kuchengetedzwa kweruzivo

Ruzivo rwamuchatipa ruchachengetedzwa neudzamu hunokosha. Zita renyu harizoziviswi vanhu mutsvkurudzo ino, uye magwaro amuchashandisa achaiswa pano kiyiwa kuitira kuti vamwe vanhu vasawane mukana pamagwaro aya.

Ndiani vamucha fonera kana muine mibvudzo kana dambudziko maererano netsvakurudzo

Fonerai panhamba idzi 0776 167 964 Dr Mugomeri kana muine kutsutsumwa, mibvudzo maererano netsvakurudzo ino.

Kana muchida kuziva kodzero dzenyu mutsvakurudzo ino kana kusabatwa zvakanaka munogona kuzivisa Africa University Research Committee yemuno panhamba idzi.

+263 20 60024 kana +263 20 60075

Kana muchibvuma kupinda mutsvakurudzo ino munoratidza nekuisa runyoro rwenyu sainecha pasi apo. Sainecha inoreva kuti;

1. Maziviswa nezvichaitwa mutsvakurudzo, njodzi dzinogona kusanganikwa nadzo
2. Mapihwa mukana wekubvudza mibvunzo.
3. Mazvipira kupinda mutsvakurudzo pasina kumanikidzwa.
4. Munonzwisisa kuti ruzivo rwatorwa runogona kuchengetedzwa panzvimbo yakakosha kuti ruzoshandiswa maererano nezveutano

Munokumbirwa kuti muise saineche yenyu pasi apo

Sainecha_____ Zuva/ Musi_____

Akuzivisai nezvetsvakurudzo iyi_____

Zuva/Musi_____

Appendix 2 (a): Gwaro remibvudzo yemutsvakurudzo

Musoro wetsvakurudzo: Status of Artemisinin resistance conferring P. falciparum kelch 13 R561H polymorphism in Mutasa District Zimbabwe**.**

Investigator: Nobert Mudare

MPH student number: 160744 Phone: 0779741234

Participant Identification Number…… **Case**

Control

Date: …….

Household Name: ……

Sex: Female ……. Male……

Age……. Village: ……

Level of education…….

Occupation…….....

1. Mishonga yeACTs inowanika nguva dzose here mudunhu rino?

   ☐Hongu ☐Kwete

2. Munotenga kupi mishonga yeACTs?
3. Kosi imwechete yemishonga yeACTs inoita mari.....?
4. Munotevedzera mitemo yenyika here pakutengesa kana kupa varwere mishonga yeACTs?

☐Hongu ☐Kwete

5. Munoshandisa mdziyo yakaita sei yekufambisa kuendesa mishonga yeACT kuzvipatara kana maclinics?

   ……

   ……

6. Mhando yemudziyo yekufambisa kutakura mishonga ine mathermometer anoshanda here?

   ☐Hongu ☐Kwete

7. Pamunotengesera varwere mishonga yemarariya munotanga mavaongorora here?

   ☐Hongu ☐Kwete

   Kana iri Kwete, chikonzero chii?.....

   ……..

8. Munoshandisa nzira yekutarisa zviratidzo zvemarariya musati mapa varwere maACTs here?

   ☐Hongu ☐Kwete

9. Ndedzipi dzimwe mhando dzinoshandiswa kurapa marariya mudunhu rino?.....

10. Munowana nguva yekudzidzisa varwere kukosha kwekupedza kosi yemarariya here?

    ☐Hongu ☐Kwete

11. Munombotenga mishonga yeACT kuvanhu vanotengesa mumisika munharaunda?

    ☐Hongu ☐Kwete

Kana mhinduro iri Hongu,

chikonzero?.....

.....

12. MaACTs amunawo aka registiwa neve National Drug Administration here?

    ☐Hongu ☐Kwete

13. Pane mutemo unochengetedza kufambiswa kwemishonga yemaACTs here?

    ☐Hongu ☐Kwete

14. Munoziva sei kuti mishonga yeACTs yamunopa varwere ndeye mhando yakatenderwa chaiyo?.....

The End

Appendix 2 (b): Participant Questionaire

Title: Status of artemisinin resistance conferring *P. falciparum* kelch 13 R561H polymorphism in Mutasa District Zimbabwe.

Investigator: Nobert Mudare

MPH student Number: 160744 **Phone:** 0779741234

Organisation: Africa University

Participant Identification Number…. **Case**

Control

Date: …….

Household Name: ……...

Sex: Female …… Male…….

Age……. Village: ……

Level of education…….

Occupation…….....

1. Is the nearest health facility within 1 km?

   ☐Yes ☐ No

2. Where do you get ACT drugs? (choose)

   I. Public Clinics

   II. Hospitals

   III. Private pharmacies

   IV. Informal community vendors

V. Shops

3. What is the average cost of 1 course of ACT drugs.....?
4. Are the ACT drugs readily available?

   ☐Yes ☐No

5. Were you tested for malaria infection before taking ACT drugs?

   ☐Yes ☐No

If no, why?

……

……..…

6. Do the staff in shops explain the importance of completing the ACT course when selling the ACT drugs?

   ☐Yes ☐No

7. Did you take ACT drugs as recommended till completing the ACT malaria treatment course ?

   ☐Yes ☐No

If No, why?.....

8. Which antimalarial drug do you prefer and why?

   ……

   ……..……..

   ……

9. Were there any educational campaigns about ACT drug adherence?

   ☐Yes ☐No

10. Did you experience side effect after taking ACT malaria treatment?

☐Yes ☐No

11. Did you receive counselling when receiving ACT malaria prescription by Pharmacists?

☐Yes ☐No

12. Have you bought ACT drugs at any given point from informal vendors?

☐Yes ☐No

If Yes,why?.....

……..

The End

Appendix 3 (a): Gwaro retenderano

Musoro wetsvakurudzo: Status of artemisinin resistance conferring *P. falciparum* kelch 13 R561H polymorphism in Mutasa District Zimbabwe.

Participant Identification Number…. **Case**

Control

Date: …….

Household Name: ……

Sex: Female …… Male…….

Age…… Village: …….

Level of education…….

Occupation…….....

1. Clinic iri pedyo nemi iri mukati mekilometer rimwe here?

   ☐Hongu ☐Kwete

2. Munowanepi mishonga yeACT yekurapa marariya? (sarudzai)

   I. Public Clinics

   II. Zvipatara

   III. MaPrivate pharmacies

   IV. Vanhu vanotengera munzira

   V. Muzvitoro

3. Kosi yemishonga ACTs inoita mari.....?

4. Mishonga yeACTs yekurapa marariya inowanikwa nyore here?

   ☐Hongu ☐Kwete

5. Makaongororwa ropa here kuti mune marariya musati mapihwa mushonga weACTs ?

   ☐Hongu ☐Kwete

   Kana mhinduro iri kwete, chikonzero chii?

   ……

……

6. Munotsanangurirwa here zvakakoshera kupedza kosi yeACTs mukurapa marariya?

   ☐Hongu ☐Kwete

7. Makamwa mushonga yeACTs nenzira inokurudzirwa nemutemo maererano nekurapa marariya ?

   ☐Hongu ☐Kwete

   Kana iri kwete, Chikonzero chii?.....

   …….

8. Ndedzipi dzimwe mhando dzemishonga dzamunoshandisa kurapa marariya mudunhu rino?

   ……

   ……

9. Munzvimbo ino makamboitwa kurudziro yekukoshesa kutora mushonga nenguva kusvika kosi yapera?

   ☐Hongu ☐Kwete

10. Makambosangana nekusagadzikana mushure mekutora mushonga weACTs?

    ☐Hongu ☐Kwete

11. Munopihwa dzidziso yekukoshesa kutora mushonga yeACTs zvakafanira nevashandi vemuma Pharmacies?

    ☐Hongu ☐Kwete

12. Makambotenga mishonga yeACTs yekurapa marariya kuvanhu vemisika kana vanotengesera munzira?

☐Hongu ☐Kwete

13. Kana mhinduro iri hongu, Chikonzero chii?.....

.....

The End

Appendix 3 (b): Questionaire Pharmacy

Title: Status of Artemisinin resistance conferring *P. falciparum* kelch 13 R561H polymorphism in Mutasa District Zimbabwe.

Investigator: Nobert Mudare

MPH student Number: 160744 Phone: **0779741234**

Organization: Africa University

Participant Identification Number……. **Case**

Control

Date: …….

Household Name: ……

Sex: Female ……. Male…….

Age……. Village: ……...

Level of education…….

Occupation…….....

1. Are ACT drugs readily available?

   ☐Yes ☐No

2. Where do you order ACT drugs?
3. What is the average cost of 1 course of ACT drugs.....?
4. Do you follow national guidelines when selling or dispensing ACT drugs?

   ☐Yes ☐No

5. What do you use to transport ACT drugs to the health facility?

   ……

   ……...…….

6. Do the trucks used for transporting ACT drugs have functional wall thermometers?

   ☐Yes ☐No

7. Do you first test patients for malaria before administering ACT malaria treatment drugs?

   ☐Yes ☐No

   If No, why?.....

8. Do you use physical examination before administering ACT malaria treatment drugs to clients?

   ☐Yes ☐No

9. Apart from ACT, what other drugs are used for malaria treatment in this area?.....

10. Do you normally explain/ counselling clients on importance of completing malaria treatment course?

    ☐Yes ☐No

11. Have you bought ACT drugs at any given point from informal vendors?

    ☐Yes ☐No

    If Yes, why?.....

    …….

12. Are the ACT drugs in stock registered following national drug regulations?

    ☐Yes ☐No

13. Is there a law enforcement regulating drug movement?

    ☐Yes ☐No.

14. How do you ensure that the drugs are not substandard of fake?.....

    ……...

The End

Appendix 4: Permission letter to submit to AUREC

"Investing in Africa's Future"

COLLEGE OF HEALTH, AGRICULTURE AND NATURAL RESOURCES

26 December 2020

To AUREC Administrator

Dear Sir/Madam

Re: Permission to Submit to AUREC for Mr NOBERT MUDARE

Reg No. 160774

Programme: MPH

This letter serves to confirm that the above-mentioned student has satisfied all the requirements of the faculty in developing his dissertation proposal and is ready for assessment.

Your facilitation for review of the proposal is greatly appreciated.

Thank you

Dr E. Mugomeri

(HOD DPHN)

Appendix 5: Permission letter to carry out the research

AFRICA UNIVERSITY RESEARCH ETHICS COMMITTEE (AUREC)

P.O. Box 1320 Mutare, Zimbabwe, Off Nyanga Road, Old Mutare-Tel (+263-20) 60075/60026/61611 Fax: (+263 20) 61785 website: www.africau.edu

Ref: AU1958/21 15 March, 2021

Nobert Mudare
C/O CHANS
Africa University
Box 1320
Mutare

RE: STATUS OF ARTEMISININ RESISTANCE-CONFERRING PLASMODIUM FALCIPARUM MALARIA KELCH-13 R561H POLYMORPHISM IN MUTASA DISTRICT, ZIMBABWE

Thank you for the above titled proposal that you submitted to the Africa University Research Ethics Committee for review. Please be advised that AUREC has reviewed and approved your application to
conduct the above research.
The approval is based on the following.
a) Research proposal

b) Data collection instruments
c) Informed consent guide
· **APPROVAL NUMBER** AUREC1958/21
This number should be used on all correspondences, consent forms, and appropriate documents.
· **AUREC MEETING DATE** NA
· **APPROVAL DATE** March 15, 2021
· **EXPIRATION DATE** March 15, 2022
· **TYPE OF MEETING** Expedited
After the expiration date this research may only continue upon renewal. For purposes of renewal, a progress report on a standard AUREC form should be submitted a month before expiration date.
· **SERIOUS ADVERSE EVENTS** All serious problems having to do with subject safety must be
reported to AUREC within 3 working days on standard AUREC form.
· **MODIFICATIONS** Prior AUREC approval is required before implementing any changes in the proposal (including changes in the consent documents)
· **TERMINATION OF STUDY** Upon termination of the study a report has to be submitted to AUREC.

Yours Faithfully
MARY CHINZOU – A/AUREC ADMINISTRATORFOR CHAIRPERSON, AFRICA UNIVERSITY RESEARCH ETHICS COMMITTEE


Nobert Mudare AUREC approval letter.pdf

DMO Mutasa

Box 1635

Mutasa

11 December 2020

BE: Permission to carry out a studvon Status of artemisinin resistance P. falciparum conferring PfKelch 13 R561H polymorphisms in Mutasa District, Zimbabwe- Nobert Mudare MPH student.

Mr Nobert Mudare ID Number 23-052200-N-23, an MPH student at Africa University has been allowed to conduct a study on Status of artemisinin resistance P. falciparum conferring PfKelch 13R561H polymorphisms in Mutasa District, Zimbabwe. The studies are solely for academic purposes and feedback is required to the DMO Mutasa before sharing findings.

Looking forward to receiving your favourable response.

Thank you

Nobert Mudare
(0779741234)

MPH student (Africa University)