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Reporting Antimalarial Resistance in Southeast Asia

Abstract *Malaria is still on the rise and affecting millions each year. The main reason is the development of resistance against the existing treatments and the requirement of the new regimen. Various areas in Southeast Asia have shown adverse health complications due to drug resistance. The transmission of malaria takes place through the Anopheles mosquito, and it is treated by antimalarial drugs that intercept the parasite's actions at different stages of its life cycle. Resistance to most drugs occurs due to genomic changes, which help the parasite survive against antimalarial drugs. It can be prevented by timely diagnosis and effective dose surveillance. No vaccine has been developed yet. Whereas newer treatments and approaches are underway.*

Key Words: Antimalarial, Resistance, Southeast Asia

Introduction

In spite of the efforts to reduce the mortality rate of malaria, it normally kills 0.5 to 2.5 million people per year. The efforts include betterment in the treatment by restricting the use of ineffective medicines, by polypharmacy and by the evolution of new drugs. By the use of current medications effectively, the mortality rate of malaria can also be reduced to a certain extent.

The spread of malaria occurs by Anopheles mosquitos that are brought about by Plasmodium protozoan. The major reason for intense malaria and death is P. falciparum. It mainly affects the erythrocytes and multiplies at a high rate in the liver. This mosquito goes to another reproduction phase before passing to another human being. They eat parasites via meal blood. The symptom of malaria occurs from 7 to 10 days after the bite of the mosquito; these include fever, headache, muscle ache, body pain. (Na-Bangchang et al., 2007)

The elevated load of P.vivax can be seen in South America and South-East Asia. There is a genetic lack of receptor expression on the boundary of erythrocytes in the people of Africa, e.g. the Duffy glycoprotein receptor, and that's why it is not present in many parts of Africa. It was suggested in a recent study that parasite is involved in using another method to invade erythrocytes. Several studies suggested that people suffering from P.vivax require immunity more rapidly to P.vivax than P.falciparum disregarding the spread intensity of malaria. However, the phenomenon that indicates the rapid adaptation to immunity against P.vivax is still unknown. In adult age, there is morbidity related to P.vivax, and older people are asymptomatic. (Gowda et al., 2018)

The Life Cycle of Anopheles

The sporozoites are small motile in nature are inoculated through blood feed by the Anopheles

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mosquito, which then enters the liver and multiple at an exponential rate. Merozoites are then produced from sporozoites within 6 to 8 days which are 10000 to 30000 in number. After that, these free merozoites enter the red blood cells when liver schizont bursts. There is increase interaction between erythrocytes and the parasites and lead to the consumption of the content of erythrocytes and changes the integrity of the cell membrane and relieve the importation of nutrients and induce haemozoin which is a toxic heam product. The parasite can enter the erythrocytes through a different mechanism like ligand-receptor mechanism. At the end of the life cycle of intraerythrocytes there become several nuclear divisions and utilization of all the erythrocyte content. After that 6 to 30 merozoites are release when erythrocytic schizont rupture and enter other red blood cells, and the cycle is repeated. (White et al., 2014)

Epidemiology

The key antecedents of the spread intensity of malaria are biting habits, mass, stability and the effectiveness of the mosquito vector. About 25 out of the 400 anopheline species are integral vectos. Anopheles gambiae are the most effectual vector that occur in Africa. They are abiding and sturdy to the seasonal changes, present in high density tropical climates, reproduce readily and favorably bite humans. Plamodium falciparum and Plasmodium vivax have around equal generality in Central America, Asia and South where transmission is little and periodic. In such type of areas, it is called entomological inoculation rate because most of the people hardly get one or two infections per year. In some parts of Ocenia and Sub-Saharan Africa the spread rate (entomological inoculation rate) of Plasmodium falciparum is 1000 per year and spread readily. In those surroundings the mortality and morbidity from malaria are marked during prompt childhood, but during adulthood most of the malaria infections are symptom free.

Stable transmission is named as year around, persistent and periodic infection. In Senegal and Sudan, the spread of malaria occurs in 3 to 4 months, and it is intense. The total protective immunity from malaria is not required where spread of malaria is focal, low and erratic (also called unstable transmission); symptomatic diseases may occur at any age. In areas where there is environmental fluctuation, social behavior (heavy rainfall along followed by drought along with malaria prevention assistance can cause epidemics along with mortality of all the groups. (White et al., 2014)

Antimalarial Drugs Available

There are seven types of drug classes available for malaria treatment. (Schlitzer.,2008). They are described briefly:

4-Aminoquinolones: This drug class include drugs like chloroquine and amodiaquine. They are involved in targeting the food vacuole and prevent the formation of toxic forms of haem. (Rathore., 2005)

Arylaminoalcohols: Includes drugs like Quinine, halofantrine, mefloquine and lumefantrine. They can treat malaria by providing schizonticidal action against intra-erythrocytic parasites of malaria (Achan et al.,2011) and can also provide gametocidal action against *P.vivax,* Quartan malaria, but are ineffective for *P.falciparum*

8-Aminoquinolones: The drug class contains solely Primaquine which is effective because it is capable of acting against the blood stages of malarian parasites (Schlitzer., 2008) primarily by inhibiting the mitochondrial metabolism of the parasite. (Nuno Vale et al., 2009)

Artemisinines: Artemisinin, a naturally occurring antimalarial agent, is extracted from herb sweet wormwood (artemisia annua). Its derivatives include artemether and artesunate. They are inhibitors of sarcoplasmic endoplasmic reticulum calcium dependent ATPase. (Rathore., 2005). Artesunate is given 2-4 mg/kg intravenous or intramuscular, whereas dosage of artemether is 3-2 mg/kg in cases of severe malaria. (White et al., 2014)

Antifolates: Antifolate drugs mainly include drugs like sulfadoxine/pyrimethamine and dapsone/chlorproguanil. The drugs like pyrimethamine and other antifolates inhibit the folate metabolism of the malarial parasite by binding to the Dihydrofolate Reductase (DHFR). (Yuthavong., 2002)

Inhibitors of Respiratory Chain: Atovaquone and proguanil are the drugs used in combination that can inhibit the electron transport chain and cause immediate reduction of the potential of the membrane in mitochondria. (Schlitzer., 2008)

Antibiotics: Antibiotics like Doxycycline and clindamycin can show antimalarial potential by inhibiting apicoplast activity (Rathore., 2005)

Drug Resistance

Infections caused by *Plasmodium falciparum* is the most fatal one among all specie and has led to most of the calamities from malaria. Through applying the usage of artemisinin combination therapies ACTs, deaths related to malaria were reduced to 20% to 30% (Halder et al., 2018). The disease burden still persists as, in 2016, 200 million new cases and 400,000 fatalities were reported. (WHO., 2016)

Before ACTs quinolones such as primaquine and mefloquine, and chloroquine were primarily employed in the treatment of malaria; they have developed resistance in different populations of the world epizootic for *Plasmodium falciparum* induced malaria, therefore, making them less effective in those areas. ACTs still remain quite successful in treatment in many parts of the world.

After 400 years, the use of quinine in malaria was documented; it still remains a useful drug in the treatment of malaria. There are certain situations like pregnancy in which quinine is the drug of choice even though its use has decreased because of the introduction of new antimalarials such as artemisinin derivatives and increased adverse drug reactions due to its use. Quine is still considered the primary drug for treatment in countries such as India and Sub-Saharan African countries. (Parija et al., 2011)

Factors Contributing to Resistance

Antimalarial resistance to *P. falciparum* takes place more often if the person is immunocompromised. One of the contributing features is drug pressure. Resistant strains can also be selected when a drug is misused like an antibiotic or used extensively. Poor quality of drugs also contributes to drug resistance. Fake mefloquine or artesunate problems were reported from almost every part of Southeast Asia. Using a drug that has extensive resistance can lead to increased mortality and morbidity rates. (Na-Bangchang et al., 2007)

Genetic Emergence of Resistance

The genetic changes that cause antimalarial resistance are very specific and uncommon and are not dependent on the type of drug used. Intra-parasitic concentrations of the drug at the target site can be affected by changes in the genome of the target site or the influx/efflux pumps regulating the site. A single genetic event can result in a series of changes, probably leading to a resistance that is both rare and spontaneous. (White NJ., 2004)

The process of resistance is divided into two phases. The first phase involves a new genetic occurrence that causes the production of a resistant strain that allows the parasite a greater chance of withstanding the effect of the drug. The second phase involves the selection and multiplication of the resistant strains to which the treatment by a specific drug or drug therapy does not remain viable. (WHO., 2000–2010)

Multidrug Resistance in Malaria

The resistance to more than two functional antimalarials is known as multidrug resistance. Healthcare workers have expanded the definition of resistance to three drugs as combining two drugs for treating malaria is a common such as sulfadoxine is always given with pyrimethamine, but this combination developed resistance due to point mutations in C59R, N511, S108M, and DHFR In which mutation in I164L causes the greatest resistance. Another combination therapy is ACTs which are currently in use as the most effective antimalarials (Ross et al., 2019). In vitro evidences of resistance to ACTs has been found in field isolates, and diminished susceptibility to these drugs has been reported recently. The main reason can be the inability of the drug to reach the target site due to active expulsion of the drug by the parasite. For example, the efficacy of chloroquine has been affected due to mutation in the PfCRT gene, but this alone can not explain the failure in treatment by aminoquinolones. The involvement of PfCRTMD1 gene (part of ABC-ATP Binding Cassette family) (Koenderink et al., 2010) also play a role in chloroquine resistance and reducing the activity of the drug at the active site.

Distribution of Antimalarial Drug Resistance in Southeast Asia

The escalating appearance of malarial parasites that present resistance to the variety of antimalarial drugs available as of today is a paramount concern, particularly when it comes to the handling of malaria induced by *Plasmodium falciparum* species in endemic areas. The situation is more prominent in Southeast Asia (SEA) owing to the aggravation of resistance to multiple drug therapy with sulphadoxine, pyrimethamine and chloroquine (CQ),

especially after the onset of failure of mono drug therapy with mefloquine (Ulehmann et al., 2005).

Lately, treatment of malaria is being restricted by arising antimalarial drug resistance. In the initial times, CQ, due to its efficacy and cost-effectiveness, was known to be the preferred drug in treating malarial infections in which the causative agents are *Plasmodium falciparum* and *Plasmodium vivax* (Dayanand et al., 2018). But nowadays, parasites have become resistant to CQ in most regions of southeast Asia (White NJ., 2004).

The first report of such resistance in *P. vivax* came out in 1989 from Papua New Guinea, which was immediately followed by the reported resistance in almost all endemic regions of Southeast Asia amongst which elevated frequency of P. vivax resistance turned out to be in Indonesian Papua coast towards the North-eastern side. (Sumawinata IW et al., 2003). Apart from Artemisinin, resistance has been reportedly developed to all available antimalarial agents so far. However, in vitro studies of resistance against artemisinin have also been lately observed. (SC Parija et al., 2011)

Demarcation of Resistance Distribution in SEA

Generally, it takes ten to fifteen years to develop resistance after the introduction of an antimalarial agent (Wernsdorfer et al., 1991). CQ, mefloquine and quinine might exhibit cross resistance since their mechanism of action is almost related. SEA happened to be the origination zone of strains of *P. falciparum* that acquired resistance to conventional drugs including CQ, mefloquine and sulphadoxine. Areas in SEA with marked P. falciparum resistance to the aforementioned antimalarial agents is demonstrated in the figure. (Kesara Na- Bangchang et al., 2007)

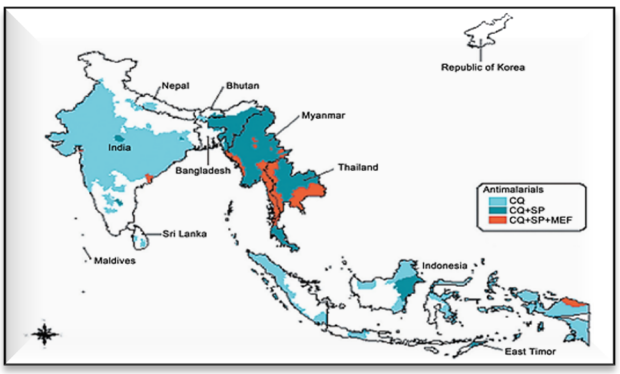

Figure 1: Shows Areas with Reported P. Falciparum Resistance to Various Antimalarial Agents

The curative response of three doses of sulphadoxine (1500mg) and pyrimethamine (75mg) has substantially decreased in a large sectors of SEA owing to the elevated resistance to these drugs. (WHO 2001a., b).

Western Cambodia and Thailand-Myanmar border came out to be the inception of quinine, amodiaquine, sulphadoxine and mefloquine resistance in the past, but as of 2007, species (P. falciparum) resistant to artemisinin have also arisen indicated by a significant delay in clearance of parasite (Dondorp et al., 2009). Thailand and Cambodian border considered to be the realm of established and possibly emerging multidrug resistance. (SC Parija et al., 2011). These regions pose a serious threat as they become the source of proliferation of resistant genes to the parts of Africa, resulting in a significant mortality rate (Nicholas J White et al., 2014).

The cause and intensification of mefloquine resistance in Thailand could be attributed to the prior excessive use of chemically derived agents like quinine and chloroquine. (Na- Bangchang et al., 2007). However, antimalarial drug resistance by species of *Plasmodium vivax* was not accounted for (Tasanor et al., 2006).

Antimalarial Resistance Status in Indonesia

East part of Indonesia indigenous to malaria has shown accelerated health complications due to drug resistance by causative agents. Far-reaching resistance shown by CQ and sulphadoxine compelled Indonesia to take on Artemisinin combination therapy (ACT) as the primary treatment option in 2004.

Investigations were conducted in the West of Sumba, Indonesia, to analyze the seasonal placement of alleles correlating with resistance amidst *P. falciparum* developed to sulphadoxine and CQ. The purpose was to aid in introducing an appropriate strategy to manage malaria in Sumba district. The results revealed a bit rising distribution of resistant alleles, particularly throughout the rainy season, which appears to be in favour of adopting ACT instead of CQ in this zone, but the findings also indicated the effectiveness of sulphadoxine either administered alone or in combination (Asih et al., 2009).

Furthermore, a study conducted in Thailand showed an interconnection between mutation due to elevation in copy number of the gene of *P. falciparum* showing multidrug resistance and the emerged resistance to the agents used in ACT, i.e., lumefantrine and mefloquine. (Price RN et al., 2004). Identical results were obtained upon examination of gene copy by PCR that demonstrated the accommodation of more than one copy in 10 percent of *P. falciparum* isolates during rainy season, whereas 25 percent accommodates in the arid season. Therefore, such analysis might be suggestive of the inappropriateness of lumefantrine and mefloquine use as ACT therapy in Sumba (Asih et al., 2009).

Mechanism of Drug Resistance on Molecular Aspects

Recent advancements at the molecular level, diagnosis of multidrug resistance parasite may help us to change monitoring strategies for resistance and as well strategies for treatments. After the Mfq therapy outcomes, the reinforcement of a gene which is known as P. falciparum multidrug resistance can be a might aid for the outcomes (Uhlemann et al., 2004). Moreover, the artemisinin targets calcium P. falciparum ATPase 6 (PfATP6). This PfATP6 is having a resemblance to sarcoplasmic-endoplasmic reticulum calcium ATPase (SERCA), which is mammalian ca^{+2}ATPase. This is why it provides us with a chance to monitor the changes for the first time in this target. Therefore, such monitoring is very critical for the best class of antimicrobial agents.

Chloroquine (CQ) and Dihydrofolate Reductase (DHFR) Resistance on Molecular Aspects

The leading therapy against malaria for many years was CQ because such a drug was effective against severe malaria (Krishna et al., 2003). This drug is given three times a day through several routes, and it's the cheapest drug, but now a day due to resistance, it is not more worthy in most places. CQ is now only used for treating vivax, malaria, or ovule malaria in South East Asia. Previously It was thought that CQ resistance was due to SNPs (single nucleotide polymorphism in PfMDR1 (Adagu et al., 1996; Foote et all., 1989). Yet, as of late, it is found that in vivo, in vitro resistance is alluded to as the PfCRT carrier, which practically manages the intraparasite concentration of the medication. Ironically phenotype CQ resistance is not associated with P. Chabaudi's and P. vivax's PfCRT mutations. The research was started in CQ resistance, and insensitive isolates continue to use the transfection process through PfCRT and PfMDR1 of CQ resistance phenotype and analyzing the contribution of SNPs. In the wake of immense hardworking in the research field, finally encoding of K76T change was found in PfCRT through laboratory transfection results in CQ resistance segregates. In Mali, such SNPs were noticed in a patient who was unresponsive to CQ therapy. The main cause of CQ resistance is the changes of N86Y in PfMDR1 and SNPs PfCRT, which are used as a bystander mutation or as a modulator (Sidhu et al., 2002). In fact, for many years, controversial CQ resistance molecular markers were N86Y in PfMDR1 and K76T in PfCRT (Adagu and Warhurst., 1999). P. glycoprotein homologue 1 is a type of P. glycoprotein encoded by PfMDR1, a member of the ABC transporter family.

The current south Asian research is conducted that how CQ is first-line therapy for plasmodium falciparum and the reason behind this was a mutation in N86Y of unlike genetically background parasite. The elimination half-life of Mefloquine is round about fourteen days, is now under drug pressure. Notwithstanding, the prevalence of K76T in certain locales of Lao, in Thailand and in Cambodia was spotted hundred precent, besides, in areas like Burma – Bangladesh borders, in southern Loa, and in Vietnam, wild-type PfCRT was noticed 40%. The folate synthesis pathway Plasmodium falciparum can be disrupted by Antifolate drugs pyrimethamine and sulfdoxine, which synergistically target the dyhydroperoate synthase and dihydrofolate reductase enzymes in combination therapy, and as a result, the parasite is killed. Resistance to this combination is already evolving the world. Resistance in pyrimethamine is explained through Plasmodium chabaudi's experiment in which the expression of gene DHFR1 is increased through amplification and rearrangement. At the S108N point, it is now obvious that here mutation directly grants resistance. The

detected adaptive advantages of mutant dhfr and dhps alleles allow prompt selection against parasites under drug pressure. Recent studies have shown a single source of resistant dhfr alleles. The most elevated recurrence of up to four mutations was seen at the Thai-Myanmar line. Conversely, in certain spaces of Laos, the extent of dhfr (>35%) and dhps (>70%) alleles are high and stayed wild sort. The correlation of quick development of resistance from Antifolate and moderate rise of resistance from 4-aminoquinolone incomparable geographic regions is because of the distinctions in their molecular mechanism of resistance. Antifolate is selected because of the mutation in their target side while CQ is selected because of changes in the transporter protein. Antifolate is chosen as a result of the mutation in their target side while CQ is chosen on account of changes in the carrier protein. This brings about the regulation of CQ -uptake and this mechanism is emerged because of the behavior of the target for CQ.

Table 1. Some Significant Polymorphisms in Drug Resistance from Antimalarial Drugs

Gene	Position	Wild type	Mutant
DHPS	436	Ser	Ala
	437	Ala	Gly
	540	Lys	Gin
	581	Ala	Gly
	613	Ala	Ser/Thr
DHFR	16	Ala	Val
	51	Asn	lie
	59	Cys	Arg
	108	Ser	Asn/Thr
	164	He	Leu
PfCRT	76	Lys	Thr
PfMDRl	86	Asn	Tyr
	184	Tyr	Phe
	1034	Ser	Cys
	1042	Asn	Asp
	1246	Asp	Tyr

Molecular Features of Artemisinin and Mefloquine

Mfq, halofantrine, and lumefantrine have a place with similar classes against malarial medications. Furthermore, Mfq was presented in Thailand as the first-line agent for treating malaria after quinine. Upgrade of PfMDR1 genes is related to Mefloquine yet not related to Chloroquine resistance. For the PfMDR1, at the point when the laboratory adopted isolates were inspected, the enhancement was related because of the increase no of mRNA expression. In actuality, because of specialized troubles in assessing the PfMDR1 copy number, thusly' such issues restricted the assay to the minuscule samples sizes, yet this required the assay like ratiomatric PCR tests. More broad sequencing research discovered non-equivalent point mutation that causes substitutes in 5 amino acid residue positions. Lately, the duplicate no of PfMDR1 is seen as the best broad pointer of treatment disappointment after Mefloquine treatment alone or in mix with As. Likewise, the increase in copy number is solidly related to high IC50 worth of basically related antimalarials like Halofantrine and Quinine, and Unrelated sesquiterpene lactone Artesunate (As) and dihydroartemisinin. Investigation SNP shows point mutation in PfMDR1 (positions of nucleotide 1042 86, and 1034) were as of late made plans to be related to Mefloquine resistance similarly as a substitute marker for copy no. In around 147 models, there was no N1246Y SNP recognized in this manner; they may have no significance in Asia southeast. (Berens et al., 2003). A more unassuming investigation was driven by using steady PCR and thereafter noticed that the extended genes duplicate a number of resistance was identified with Mefloquine and Artemisinins (Pickard et al., 2003).

In the southeast of Asia, for a long time during the Mfq pressure, an outstanding abatement in the recurrence of N86Y was seen, in spite of this, the recurrence of K76T mutation in PfCRT was extremely high, yet the real separation recurrence (about 90%) mutation in these two carriers can be found in the African context. This perception underscores the chance of "interfacing" mutations in various medication carriers that offers resistance from antimalarial drugs. Such collaboration is by situating PfMDR1 and PfCRT in the food vacuoles of parasites and can be more conceivable in a different body

(Fidock et al. 2000). Over an earlier decade in southeast Asia, treatment routine of articiminin based-mix have been deliberately presented, which is profoundly successful, all around endured and can give security hypothetically against the rise of resistance and furthermore lessen the transmission by forestalling the advancement of gametocyte. So because of these highlights (high treatment viability and justification for what it is utilized) can diminish the frequency of Falciparum malaria in southeast regions of Asia.

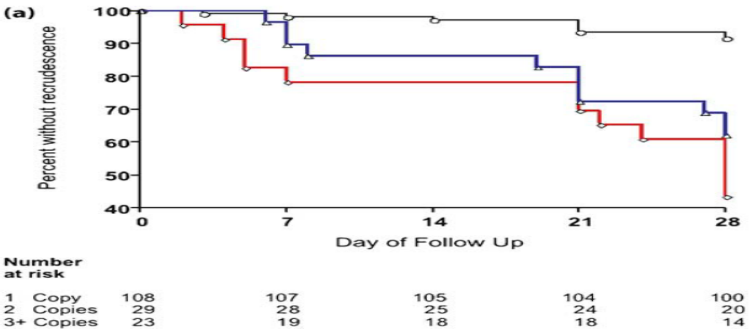

Figure 1

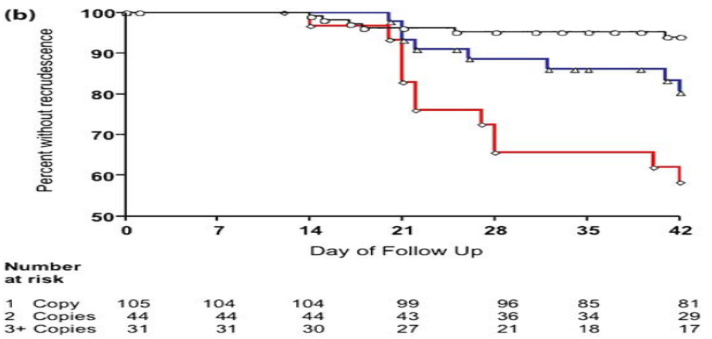

Figure 2: Total percentage of a patients who are liberated from the Malaria after the therapy with (a) monotherapy of MEFLOQUINE or (b) MEFLOQUINE and ARTESUNATE 3 days therapy (black =>one copy, blue => two copies, and red => more then three).

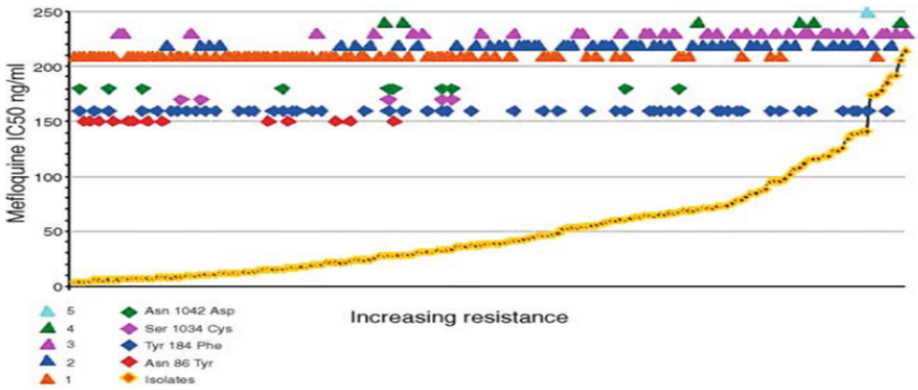

Figure 3: Mfq IC_{50} Distribution by Numbers (Triangle) and Codon Mutations (Diamond)

PfATP6, a ca^{2+}ATPase which is the target site for the artemisinin derivatives, has been recently identified. Amino acid N86Y heterogeneity was recognized as separate full-length sequencing with moderately high As IC50 values in m2 and m1 area of ATPase. This polymorphism exceeds 100 Thai isolates with a wide span of IC_{50} values (0.26–23.5 ng/ml); be that as it may, there was no correlation established among the higher IC50 value for As and this polymorphism. (price et al. 2004). The way that the affectability to As and Mfq are both balanced together by the molecular mechanism is by all accounts stressing. Nonetheless, the oddity is that assuming PfMDR1 articulation expands the yield of antimalarial medications, for example, artemisinin, this can lessen the opportunity of choosing artemisinin-resistance PfATP6 variations by keeping up low determination tension on this objective. From the outset, both As and Mfq target is limited to various sides of a similar film, for example, the Mfq meddle with hemoglobin food vacuole though As follows up in PfATP6 which is a layer in the cytoplasm of parasites. In any case, numerous insights regarding the layer association are as yet missing (Functionality and unequivocal quality) between the SERCA type construction of the cytoplasm of parasites and the film segments of food vacuoles. These perceptions obviously bring up the theory that is appropriate for the exploratory affirmations.

In Vitro Test

The in vitro tests were designed for the detection of drug resistance by estimating the inherent responsiveness of *Plasmodium falciparum* to antimalarials. After exposure of parasites to a particular quantity of the drug, hindrance of development to schizonts is noticed. (Organization Mondale De La Sante., 2010) In 1922 Bass performed the first assay to check the antimalarial drug action on, in vitro cultured human malarial parasite. To illustrate the schizontocidal effects, isolated parasite was exposed to single concentration of Quinine during a 29 hours incubation period. To combat the problem of drug resistant parasite a procedure named **macrotest** or **macrotechnique** was introduced by Rieckmann. Its purpose was to note the expansion of chloroquine-resistant P. falciparum across the globe. A conventional testing kit and strategy developed by WHO regarding macro testing was extensively used by field students in different countries in the middle of 1976 and 1987. It was simple to use but was rejected in late 1980s because of drawbacks. Every single present analyses are established in accordance to the in vitro culturing technique introduced by Jansen and Traeger. Researchers also found that it can be used for the estimation of reactivity of parasites to both present and new drugs (Basco., 2007).

Names of Tests

Advanced assays were established for newer antimalarial drugs that were effective against the resistant parasites and also due to the diminishing efficacy of quinine in South East Asia. (Basco., 2007) Following is the list of in vitro methods to assess the resistance: WHO in vitro micro test, Isotopic micro test, Colorimetric assay, Lactate dehydrogenase assay, Histidine protein rich assay (Parija and Paraharaj., 2011).

Advantages and Limitations

In vitro assays are beneficial in many ways. These are helpful in: studying the actions of several drugs at a time, together with the drugs that are still in the investigational phase. Studying the drug interaction when multiple drugs are used for treatment. Identification of cross resistance to individual drugs. (Basco., 2007)

Besides advantages, in vitro assays also have some limitations. The difference in results is due to the contrast among laboratories. Variation in results also occurs due to a lack of adherence to the standard protocols. Intrinsic systemic delicacy due to existence of several other drugs in the blood circulation can be the other reason. (Organization Mondale De La Sante., 2010)

Molecular Markers

Resistance to antimalarial therapy can also be identified by molecular or genetic markers. By knowing the genetic changes linked to resistance, specific drug resistance can easily be verified by molecular approaches.

Genetic Markers of Resistance

Genes that are responsible for drug resistance due to mutations in them are dihydrofolate reductase, dihydropteroate synthase, chloroquine resistant transporter, Pfmdr-1 and cytochrome b. (Organization Mondale De La Sante., 2010)

Advantages and Limitations

Encouraging points of the molecular marker method are: On the filter paper strips, blood samples can be

collected and stored for a longer duration. Specimens can be gathered, carried and stocked. Different samples can be studied within a brief time period.

Challenges that make this method less effective are contrasting methods of varying reactivity are used in laboratories. If identifying method is not specific, then it may lead to the masking of a sensitive populations. The close association between National Malaria Control Programmes and research institute is another demanding task. (Organization Mondale De La Sante., 2010)

Preventing the Resistance to Antimalarials

Irrational use of antimalarial agents, which includes improper dose administered to the patient such as subtherapeutic levels of the drug in plasma, may cause the development of mutations in the parasites. Moreover, not completing the required duration of treatment is another major factor for emerging resistance to antimalarials. Optimization of the dose of the drug and monitoring the duration of treatment may prevent the emergence of resistance.

Identification of illness in the initial stages followed by immediate treatment may be effective in combating drug resistance in patients with malaria. Health education focused on limiting parasite exposure and preventing antimalarial resistance must be provided to the high-risk population. These measures should be implemented on a routine basis along with efficient diagnosis and disease surveillance. In addition to this, the dispensing of antimalarials must be limited, and their use must be controlled. The drug therapy against malaria in patients should be monitored timely to ensure whether the current treatment strategies are effective and if any change may be required. Monitoring is essential to indicate the alarming chances of drug resistance that might occur, along with observing the resistance to insect killers. Drug resistance is identified by four parameters that include: response of drug therapy indicating failure to treatment, the amount of drug present in plasma, the molecular marker of parasite and the parasite sensitivity assessment determined through in-vitro testing. Currently, no vaccine against malaria is available, so research on malaria should be encouraged contributing to the development of vaccines, new antimalarial drugs targets, and research is also being carried out to develop genetically engineered transgenic vectors that are ineffective in the transmission of the parasite (Na-Bangchang et al., 2007)

Newer Antimalarial Agents

With the emergence of resistance in parasites to existing antimalarial drugs (e.g., quinine, chloroquine), there is a need to explore newer antimalarial agents in order to overcome the antigenic variation in the parasite. New drugs can either be isolated from plant materials by applying bioassays for malarial parasites in-vitro and in-vivo using any model animal (mice), or these can be synthesized based on biochemical and antigenic variation in the parasite.

Artemisinin Combination Therapies

Where *P.falciparum* is resistant to chloroquine, artemisinin combination therapies recommended by WHO guidelines are advised for the treatment of uncomplicated malaria in endemic regions (Dondorp et al., 2009), these combinations have been found to be effective in limiting chances of resistance to antimalarial agents. The extent to which the parasite is resistant to the partner drug determines the choice of a combination drug with artemisinin (WHO., 2015). Since artemisinin, along with its derivatives has shorter half-lives, they are combined with drugs having longer half-lives to ensure a prolonged antimalarial effect. Some of these combinations as per WHO are amodiaquine-artesunate, mefloquine-artesunate, artemether-lumefantrine, and sulfadoxine pyrimethamine-artesunate (Parija and Praharaj., 2011).

New Drugs in Clinical Use

Artemether-lumefantrine (co-artemether/riamet) used clinically for treating uncomplicated type malaria is a synergistic combination in which the potential target of both drugs is the detoxification of hem in food vacuoles (Weisner et al., 2003). Another synergistic combination currently used for treatment as well as prophylaxis of malaria is that of atovaquone and proguanil (malarone). Although proguanil has low efficacy as compared to its derivative (cycloguanil), it has a significant synergistic effect when used with atovaquone through inhibition of the electron transport chain of mitochondria. But the use of this combination has documented alteration in the gene of cytochrome b resulting in mutation and, ultimately, resistance (Filver et al., 2002).

New Antimalarials in Clinical Development

These include lap-dap (chlorproguanil with dapsone), tafenoquine, pyronaridine, and fosmidomycin. Chlorproguanil-Dapsone is in clinical development as a substitute for sulfadoxine-pyrimethamine because

resistance to the former combination developed relatively slowly as compared to the latter. Tafenoquine is a derivative of primaquine having longer half live and less toxicity. It targets the erythrocytic phase of the parasite by inhibiting polymerization of heme. It is also active against hepatic stages and gametocytes. Pyronaridine is a 4-amino quinolone derivative that has only been developed and registered for use in China since the 1980s; it is used against parasites resistant to chloroquine. Fosmidonycin inhibits the activity of enzyme reductoisomerase deoxy xylulose phosphate (DOXP) present in the apicoplast of the parasite and involved in the mevalonate independent pathway (Jomaa et al., 1999).

Other biochemical drug targets currently in preclinical development include a spiroindole (cipargamin) - acts on PfATP4 altering the homeostasis of sodium, targets the intraerythrocytic stage of parasite (Zhang et al., 2016); albitiazolium - inhibits the uptake of choline which the parasite requires to synthesize its membrane via production of phosphatidylcholine; Methylene blue – heme polymerization inhibitor targets the glutathione reductase enzyme of parasite P.falciparum; leupeptin – a protease inhibitor; Di sesquiterpene gossypol – inhibit glycolysis via inhibiting the parasite's lactate dehydrogenase (Weisner et al., 2003); thiolactomycin and triclosan derivatives – fatty acid synthesis inhibitors; artefenomel – a peroxide clearing parasitemia in malaria caused by P.vivax and P.falciparum; chymostatin – a serine protease inhibitor which inhibits the invasion of erythrocyte; a combination of fosmidomycin with piperaquine – inhibition of the pathway DOXP (Shibeshi et al., 2020).

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