

Full Length Research Paper

# ***In-silico* studies of multi drug resistance (MDR) genetic markers of the *Plasmodium* species**

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Multi-drug resistance malaria species has been and still is the cause of much morbidity and mortality of malaria throughout the tropics. This epidemic has devastated large populations likewise posed a serious barrier to economic growth in developing countries. The major obstacles however, is its prevention and treatment due to emerging multi-drug resistant (MDR) species. Therefore, anti-malarial drug development needs to continue so that novel and highly effective anti-malarial can be plugged into recommended strategies and vaccine development. The sequencing of the various MDR genes of *Plasmodium* has contributed tremendously to the understanding of the MDR malaria parasites. The current research therefore, engaged the use of an in-silico approach to seek the strategies in analyzing as well as offering some likely solutions to malaria therapies. Four *Plasmodium* species: 2 from rodents (*Plasmodium chabaudi* and *Plasmodium yoelii*) and 2 from human (*Plasmodium vivax* and *Plasmodium falciparum*) multi drug resistance genes were compared using bioinformatics tools. The phylogenetic relationships and species identification of the MDR genes of the parasites were downloaded from web base resources and performed as confirmed by the ClustalX programs. The results showed a variation in the up/down stream algorithms alignment of their phylogenetic relationships. This therefore, showed that some resistance genes within a population may vary within the same drug. The results showed a significant difference of  $p < 0.001$  with a 95% CI. Through these efforts, our goal was to better understand how drug resistance occurs. This knowledge therefore, will facilitate the rationale to design new effective as well as check the emerging of multi-drug resistant *Plasmodium* strains.

**Key words:** In-silico, comparison, multi drug, resistance genes, *Plasmodium*.

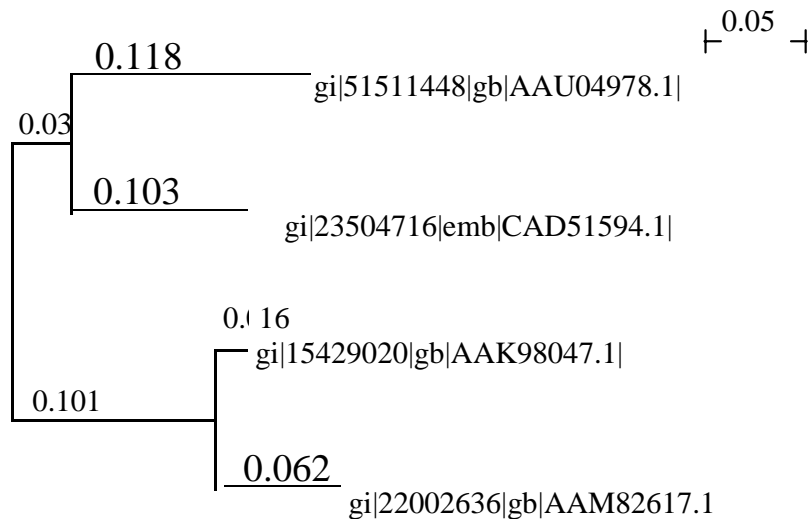
## INTRODUCTION

Multi-drug-resistant *Plasmodium* species is a major public health problem worldwide (Rijken et al., 2008; Karyana et al., 2008). However, substantial progress has been made in the last decade to reveal the underlying mechanisms of drug action and resistance. The current emergence of multi-drug-resistant (MDR) plasmodia species menace threatens to reverse these efforts. This is due to an increased exposure of *Plasmodium* species to sub-lethal doses of anti-malarial drugs. Apart from that it has placed the populations that are routinely in contact with malaria-transmitting mosquitoes and those with limited access to

health services at risk to malaria attack (Rockman and Kruglyak, 2006; Emiliana et al., 2008). If steps are not taken to address the root causes of drug resistance, the drugs will also lose their effectiveness in the near future.

However, according to Carlton et al. (2004) the arrival of the genomics and bioinformatics revolution, comparative genomics has scaled up the whole genome comparisons which are now used to describe relative genome composition, genome organization, identify orthologous and paralogous genes. This has given a clear picture of classifying species-specific genes and the evolution of organisms, in all three domains of life: bacterial (Fraser et al., 2000), archaeal (Nelson et al., 2000) and eukaryotic (Rubin et al., 2000). However, the comparative genomics of plasmodia genomes still is a discipline in its understudy (Carlton et al., 2004). The

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**Figure 1.** Phylogenetic tree of the MDR gene sequences of the 4 *Plasmodium* species from GenBank using similarities.

current research therefore, engaged the use of in-silico approach to compare MDR genes of *Plasmodium* species downloaded from various resourced data bases. The work will focus primarily on the MDR genes of rodent malaria parasites (*P. yoelii* and *P. chabaudi*) and comparative MDR genomic studies with the human malaria species (*P. falciparum* and *P. vivax*), since these are the most advanced. This will give us the insight of the sequence analyses which might be of help to malaria therapies.

The human MDR (P-glycoprotein) gene family is known to include two members, MDR1 and MDR2. The product of the MDR1 gene which is responsible for resistance to different cytotoxic drugs (multi drug resistance) appears to serve as an energy dependent efflux pump for various lipophilic compounds (Chin et al., 1989). Multi drug resistance in human and rodent cell lines is associated with decreased intracellular drug accumulation and correlates with the increased expression of MDR genes which encode membrane glycoprotein (P-glycoprotein) of approximately 170 kilodaltons. Expression of a single human gene designated MDR1 or its rodent counterpart is sufficient to confer multi drug resistance phenotype to drug- sensitive cells. However, the molecular bases for multi drug resistance in plasmodia are still not fully understood. An association between *P. falciparum* pfmdr1, which encodes a trans-membrane glycoprotein (Pgh1, for Pglycoprotein homologue 1) and the multi drug- resistant (MDR) phenotype was first reported in 1989 (Foote et al., 1989). Point mutations in pfmdr1, most notably at codon 86, have been associated with decreased chloroquine sensitivity (Price et al., 1999; Agbonlahor et al., 2008). Furthermore, in a study involving genetic cross, chloroquine resistance was found to segregate with cg2 (located on chromosome 7) rather than with pfmdr1 (located on chromosome 11) (Su et al., 1997). Amplification of the pfmdr1 gene copy number is

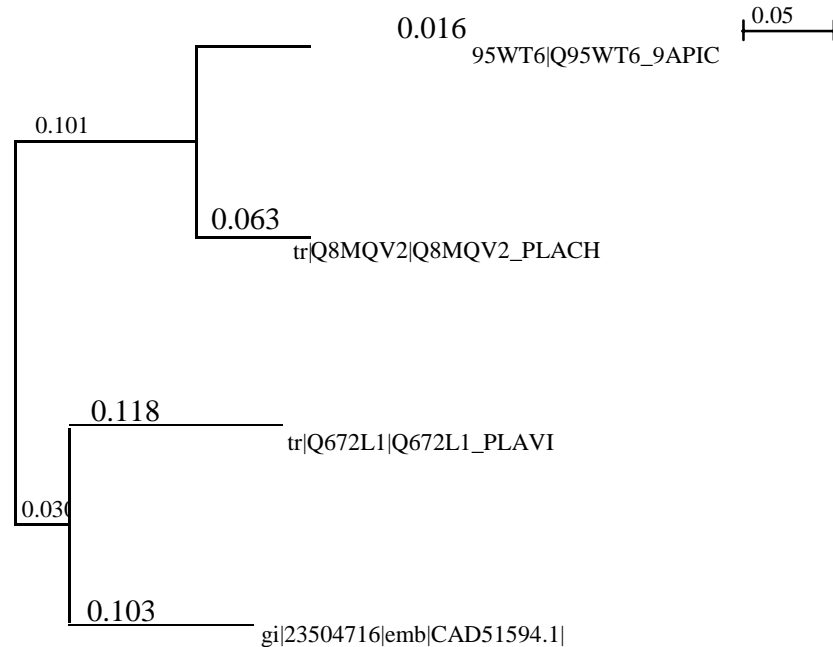
associated with resistance to mefloquine and halofantrine, both in laboratory and field isolates (Wilson et al., 1993). In *Plasmodium*, mdr homologues encoding P-glycoprotein-like molecules have been proposed as determinants of malaria drug resistance and associations have been reported between chloroquine resistance and amplification or mutation of the mdr -like gene pfmdr 1, which encodes Pgh1 (Reed et al., 2000)

## MATERIALS AND METHODS

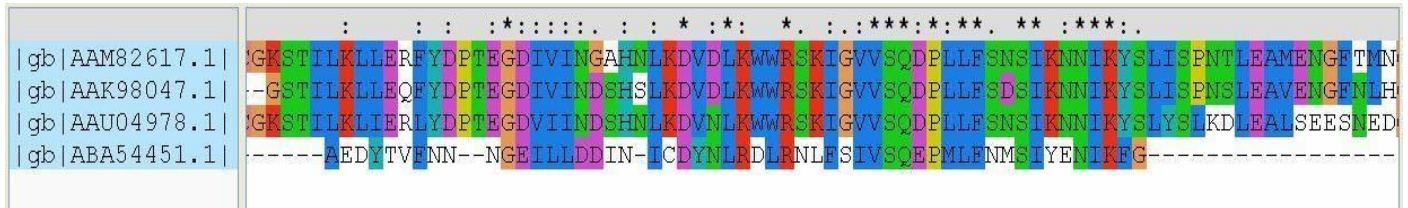
Sequence encoding for multi drug resistances in 4 *Plasmodium* species: 2 from rodents (*Plasmodium chabaudi* and *Plasmodium yoelii*) and 2 from human (*Plasmodium vivax* and *Plasmodium falciparum*) were downloaded from GenBank (USA) and ExPASy (Swiss). The algorithms of nucleotide-nucleotide BLAST and protein-protein BLAST of protein sequences were obtained from the above databases and done using the fasta format. A comparative homology study of the 2 MDR genes of rodent malaria parasite sequences and 2 from human were done. The multiple sequence alignment algorithms of the multidrug resistance genes of these plasmodia were done using ClustalX (ver 2.0.9). The plot of trees were obtained from amino acid sequences of the multi drug resistance genes of the malaria parasites with the help of "drawgram" program of the PHYLIP package (ver. 3.6) which was downloaded from <http://evolution.genetics.washington.edu/phylip.html>. All the databases and software used in the studies were supplied by Wellcome Trust, UK and are currently available on the world-wide web.

## RESULTS

Multiple sequence algorithms alignment of the 4 MDR resistance genes has depicted the presence of the conserved sequence regions and their relationship as shown in Figures 1 and 2. The results showed a significant difference of ( $p < 0.001$ ) with a 95%



**Figure 2.** Phylogenetic tree of the MDR gene sequences of the 4 *Plasmodium* species from ExPASy using similarities.



**Figure 3.** Multiple sequence alignment of MDR genes encoding nucleotide of the 4 *Plasmodium* species obtained from GenBank.

confidence limit (CI) among all the MDR genes studied. The results in Figure 1 showed that the genealogy of the *Plasmodium* species MDR orthologue gene sequences from the GenBank were variable. There was no total homology in the encoding genes as can be seen in their branch lengths ( $p < 0.001$ ). The branch lengths of the human's parasites were 0.103 (*P. vivax*) and 0.118 (*P. falciparum*) respectively while the one from rodent parasites were 0.016 (*P. yoelii*) and 0.062 (*P. chabaudi*) respectively. There was a similarity between the two *Plasmodium* rodent species as well as between the two *Plasmodium* human parasites (Figure 1).

The results in Figure 2 show the 4 *Plasmodium* species MDR gene sequences from ExPASy resourced web base. The branch lengths for the human *Plasmodium* species were the same as those obtained from GenBank (0.103 and 0.118 respectively) while the one from rodent parasites were 0.016 (*P. yoelii*) and 0.063 (*P. chabaudi*) respectively (Figure 2). The results also showed a great similarity between the 2 *Plasmodium* rodent species as against the 2 *Plasmodium* human parasites (Figure 2).

The phylogenetic tree showed that the 4 *Plasmodium* parasites were from the same genus as shown from their branch lengths of 0.030 and 0.101 (Figures 1 and 2) indicating that great sequence homology.

The results of Figure 3 showed the multiple sequence alignments encoding the amino sequences of the 4 *Plasmodium* parasites from GenBank. Almost the entire sequence from the 4 *Plasmodium* species could be found aligned. The residues in the sequences also showed a slight varied homology with close variation as shown in the phylogeny results. The same results were obtained from ExPASy as shown in Figure 4. The multiple sequence alignment encoding the amino sequences of the 4 *Plasmodium* parasites showed their high level genus sequence homology (Figures 3 and 4).

## DISCUSSION

Plasmodia parasites isolated from vertebrates have been shown to exhibit a wide range of phenotypic variations,



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