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Changing *Plasmodium falciparum* genotypes during long term and short time culture in drug free media

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Parasite culture assay is an important tool for malaria drug resistance surveillance. This assay usually leads to the large-scale production of cultured parasites. Consequently, the nature and longevity of parasite genotypes are monitored without influence from the host factors. Here, we set out to study the genotypic and phenotypic dynamics and stability of field isolates adapted in continuous cultures. Three field isolates collected from patients presenting with uncomplicated malaria in high transmission area were maintained in drug-free continuous culture media period spanning 90 days. Aliquots picked at intervals of 24-48 hours gave 56 samples from each of the isolate within the 90 days period. Each aliquot was regarded as a separate parasite sample and genotyped using 12 microsatellite (MS) markers. Further, single nucleotide polymorphism (SNP) analyses of 23 drug resistance markers were done. The 50% inhibitory concentrations (IC50) against four antimalarial drugs were estimated in some of the samples at aliquoting time-points that coincided with parasitemia levels greater than 3%. Samples from each patient (parasite-line) were compared as they were passed through the continuous culture. Data revealed genotypic and phenotypic profiles for the three parasite-lines fluctuated from one generation to the next with no specific pattern or periodicity. Multilocus analysis revealed that of the three parasite-lines showed genetic diversity and structure. SNP/MS changes occurred simultaneously in the parasite generation. The mean IC50 for the four drugs tested in the three parasite lines changed significantly from generation to generation. Our study revealed parasite genetic and phenotypic characteristics fluctuates in short-and long-term cultures, which indicates that parasite genetic information obtained even in short cultures is likely to be different from that of the natural infection parasites. These findings endorse ex vivo analyses of parasites in real-time is important in formulation of anti-malaria drug policies.

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