In vitro sensitivity to antimalarial drugs and polymorphisms in *Pfg377* gene in *Plasmodium falciparum* field isolates from Mewat, India

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ABSTRACT

The malaria infection is the interplay of several complex factors, among which drug resistance and gametocyte biology are the crucial ones. Measurements of the drug sensitivity assays and prevalence of gametocyte genes play a major role in malaria eradication programmes. The purpose of this study was to assess the current drug sensitivity profile in the field isolates and type the *Pfg377* gene. *In vitro* drug sensitivity assay was used to assess the susceptibility of *Plasmodium falciparum* field isolates collected from Mewat (Haryana), located in Northern region of India to four anti-malarial drugs. The inhibitory concentrations (IC_{50}) for the four drugs viz Chloroquine (CQ), Artesunate (AS), Sulphadoxine (SD) and Pyrimethamine (PYR) were in the range of 10.11–113.2, 2.26–4.08, 13.31–43.91 and 0.76–4.91 nM respectively in the evaluated 25 field isolates. The *Pfg377* allele typing in the 26 field isolates revealed different types of haplotypes (A, B, C and D) varying by the size and number of repeats and deletions. The *Pfg377* allelic types in the cryopreserved and adapted isolates of *P. falciparum* were compared to determine the parasite lines in them. The allele types or presence of different clones in the same sample could not be correlated significantly with resistance to any of the four drugs tested in the study. Our study revealed different drug sensitivity profiles of field isolates from Mewat region and allele typing of *Pfg377* gene revealed different haplotypes of field isolates were circulating in parasite population.

KEYWORDS

P. falciparum; *in vitro* drug sensitivity; *Pfg377* gene; polymorphisms

Introduction

Drug resistance is a major challenge in containing the malaria infections worldwide and the spread of multidrug resistant *falciparum* malaria has led to the adoption of Artemisinin Combination Therapies (ACT) in malarious regions [1,2]. The malaria incidence in India is about two million per annum though reports indicate that 9–50 times malaria cases go unreported with several malaria endemic regions in the country [3,4]. Various sensitivity test systems have been developed for sensitivity monitoring the drug sensitivity of *P. falciparum* field isolates [4]. The most commonly used methods for drug sensitivity assessment are *in vitro* tests based on the development of schizont maturation inhibition (SMI), incorporation of radiolabeled precursors, histidine-rich protein II (HRP II) or enzymatic activity of parasite lactate dehydrogenase (pLDH) [5]. The *in vitro* sensitivity test based on the standard micro-technique recommended by the World Health Organization (WHO) using the SMI test has been in vogue worldwide in most of the highly multi-drug-resistant regions [6].

In India, the CQ resistance in *P. falciparum* was reported in 1973 from Assam for the first time and later from other parts of the country [7,8]. In the year 2005 in India the national malaria control programme introduced ACT as the first line of treatment for *falciparum* infections. Currently, the recommended ACT by the national program is artesunate with sulphadoxine (AS+SP) across the country except for north-eastern states where the recommended ACT is combination of Artemether–Lumefantrine (AL) [9]. The reports of artemisinin resistance from other parts of the world emphasizes on the need to routinely carry out the drug sensitivity assays of the field isolates. Although the artemisinin resistance is not yet reported from the country but it is necessary to monitor closely for any early development of reduced susceptibility to the drugs used in the ACT. The *in vitro* data not only yields the important information about the drug response but also reveals the existing and emerging trend of drug resistance in parasite population.

The information available on gametocytogenesis in natural infections is limited till date [10]. Osmiophilic bodies found predominantly in female gametocytes

Figure 1. Map depicting the Mewat region, Haryana located in northern part of India the sample collection site for *P. falciparum* field isolates.

are involved in their escape from erythrocyte after the macrogametes are ingested by mosquito during blood meal [11]. The only gametocyte-specific protein associated with osmiophilic bodies is *Pfg377*, encoded by a single exon of 9360 bp on chromosome 12 [11]. *Pfg377* is a gametocyte specific gene, with polymorphic region 3 used for allele typing which by PCR reveals the predominant allele present in the parasite population [12]. There have been reports indicating that some correlation might exist in drug sensitivity and gametocytemia in natural infections [13]. The seasonal transmissions of malaria involving the sub-patent infections are not

understood properly and to understand the complex gametocytogenesis process the analysis of gametocyte gene *Pfg377* involvement is crucial [14,15]. Till date, the information available on prevalence of gametocyte producing clones is limited in Indian context too [16].

The knowledge of drug sensitivity profile and parasite diversity is critical for the success of malaria intervention programmes. We studied the current pattern of drug sensitivity in *P. falciparum* field isolates to four antimalarial drugs. We also determined the existing polymorphisms in the *Pfg377* gene in field isolates for identifying probable resistant genotypes with multiple clones.

Materials and methods

The samples collected were from the patients living in and around Mewat, Haryana region between the months of August–September 2015 (Figure 1). The ethical clearance of the study was given by the Institutional Ethics Committee (IEC) (ECR/NIMR/EC/2011/108). The aims and objectives of the study were explained to the patients and blood samples were collected only from the informed consenting patients. A total of 200 oil emergence fields were examined before the smears were labeled negative or positive. After diagnosis of the infection by microscopy and rapid diagnostic tests (RDT), with no history of prior drug treatment, the confirmed blood samples were selected for this study. The diagnosed positive cases of falciparum malaria were treated with ACT as per the national policy [8]. About 2–3 ml of venous blood from confirmed patients was collected and 100 µl of the sample was used to make filter spots on Whatman paper (No.3). Plasma and buffy coat were removed from the samples by centrifugation and the packed Red Blood Cells (RBCs) were cryopreserved and transported back to the laboratory for culture studies and *in vitro* drug assays.

The consenting patients from whom the blood samples collected were >7 years of age (in case of minors the consent was taken from guardians) and came to the health centre for treatment with symptoms of malaria. In the lab the cryopreserved samples were revived in the RPMI 1640 culture medium supplemented with gentamicin [0.01 mg/mL], 25 mM HEPES buffer, 25 mM NaHCO₃ and 5% human serum maintained in 5% CO₂ with incubation at 37 °C. The growth of the parasite *in vitro* was visualized daily by microscopic examination of the Giemsa stained blood films.

The drug plates were prepared in pmole/litre as per the WHO *in vitro* microtest procedure and the assay was done in duplicate according to WHO in a 96-well microplate based on SMI [17]. CQ, AS, SD and PYR were obtained from Sigma-Aldrich and stock solutions of 10 mM concentrations were prepared in ethanol and aliquoted into eppendorf tubes at –20 °C until further use. The stock solutions were diluted in two fold dilutions to working stocks for CQ (1–64 pmol/well), SD (10–10,000 pmol/well), PYR (0.125–1250 pmol/well) and AS (0.15–150 pmol/well). Modified RPMI 1640 medium with no PABA was used for SD and PYR *in vitro* assays [18]. The 96 well culture microtest plates were predosed with ascending concentrations of drugs to obtain nine final dilutions with controls set up for all the four tested drugs. The packedRBCs of collected samples were washed thrice in wash media and resuspended to make 3% cell suspension in the complete medium. A 50 µl of the blood media mixture (BMM) was added in the pre-dosed drug plate in triplicate with serial drug dilutions (down the plate) and then incubated in CO₂ gas mixture at 37 °C for 24–30 h for schizont maturation. After 24 h incubation, a thin smear

was prepared from the control well to observe mature schizonts and if more than 10% schizonts (with more than three nuclei) were seen in the control wells, it was considered to be valid. The thick smears were prepared and after Giemsa staining the schizonts were counted out of a total of 200 asexual parasites. For the present study, the 3D7 (sensitive strain) and RKI-9 (resistant strain) were included as internal controls for *in vitro* assay.

Genomic DNA extraction of *P. falciparum* samples was done from filter paper blood spots using QIAamp DNA Blood Mini Kit according to the manufacturer's instructions with slight modifications (Qiagen Inc). At least four punches of same dimensions (4 mm approximately) were taken from filter spots and incubated overnight at 4 °C for maximum DNA yield. After the DNA extraction nested PCR assay with 18S rRNA primers was performed to differentiate between the mixed infections of *P. vivax* and *P. falciparum* [19]. The single *P. falciparum* infections after the species specific PCR were only considered for further molecular analysis. The repetitive polymorphic regions in different allelic families of *msp1* (block 2–K1, MAD20, RO33) and *msp2* (block 3–FC27, 3D7/IC) genes were amplified by PCR. These alleles have conserved regions flanked by repeat sequences of variable regions [20]. The primary PCR amplification of *msp1* and *msp2* genes comprised of an initial step of 95 °C for 5 min followed by 30 cycles of 95 °C for 1 min, 58 °C for 2 min, 72 °C for 2 min and a final extension of 72 °C for 5 min. The PCR products for *msp1* and *msp2* were separated on 1.5% Ethidium bromide stained agarose gels for visualization under UV illumination.

Among the four regions of *Pfg377* gene, region 3 is the most polymorphic region which encodes seven degenerate amino-acid repeats; alleles of this gene thus vary by multiples of 21 base-pairs [21]. The presence of high polymorphism existing in the 3 region of this gene is used as molecular marker to study the parasite diversity. PCR primers and conditions for amplification of region 3 were as described previously, with minor modifications [22]. PCR products for *Pfg377* were separated by electrophoresis in agarose gels and were sized. The nested PCR products for region 3 of *Pfg377* were purified using QIA quick PCR purification kit (QIAGEN) according to manufacturers' instructions and checked for purity in 1.5% agarose gels. The PCR products were sequenced using the cycle sequencing reaction with ABI Big Dye terminator version 3.1 before separation of reaction products on an ABI 3730 DNA analyzer. The *Pfg377* gene of 3D7 strain (genID: Pf 3D7_1250100) was used as reference for sequence comparisons of the field isolates. These sequences were assembled, aligned and edited using MEGA 6 and Finch TV program [23].

The IC₅₀ of field samples for all the four drugs was determined using HNonLin V1.1 software by non-linear regression analysis [24]. The cut-off values for

Table 1. Details of field isolates showing parasitaemia at the time of sample collection.

S.No.	Isolate No.	% Parasitaemia	In vitro adaptation	Type of infection
1.	MWT 1	0.82	+	
2.	MWT 2	2	+	
3.	MWT 3	1	+	
4.	MWT 4	0.78	+	
5.	MWT 5	1.2	+	
6.	MWT 6	0.79	+	
7.	MWT 7	3.7	+	
8.	MWT 8	0.69	+	
9.	MWT 9	0.71	+	
10.	MWT 10	0.79	+	
11.	MWT 11	1	+	
12.	MWT 12	0.65	+	
13.	MWT 13	0.76	+	
14.	MWT 14	2.2	+	
15.	MWT 15	1.3	–	Mixed infection
16.	MWT 16	0.4	+	
17.	MWT 17	1.3	+	
18.	MWT 18	1.7	+	
19.	MWT 19	0.9	–	Mixed infection
20.	MWT 20	1.3	+	
21.	MWT 21	0.45	+	
22.	MWT 22	1.2	+	
23.	MWT 23	1.5	–	Mixed infection
24.	MWT 24	0.4	–	Mixed infection
25.	MWT 25	0.65	–	
26.	MWT 26	0.46	–	
27.	MWT 27	2.4	+	
28.	MWT 28	0.3	–	
29.	MWT 29	1.3	+	
30.	MWT 30	0.8	+	
31.	MWT 31	1.5	+	
32.	MWT 32	2.3	+

Figure 2. The logarithmic means of IC₅₀ value for the tested drugs –Chloroquine (CQ), Artesunate (AS), Sulfadoxine (SD) and Pyrimethamine (PYR) in the field isolates. Statistical analysis was performed by *t*-test one way ANOVA and their *p*-value against all antimalarial drugs < .05 is significant. (*t*-test value for the different drugs are: CQ- 2.8, ART- 1.12, SD- 5.67 and PYR- 1.06)

determining antimalarial drug sensitivity were generated and their IC₅₀ values determined. The IC₅₀ value is the cut-off where the drug inhibits 50% of schizont maturation as compared with the development in drug free controls. The Bonferroni correction was used to control for the increased probability of false positives in multiple corrections. Expected heterozygosity index (H_E) which measures locus diversity was calculated using the formulae $H_E = [n/(n-1)] [(1 - \sum P_i^2)]$, where n = sample size, P_i = allelic frequency [25]. The correlation between the IC₅₀ values of the drugs with polymorphism in *Pf*g377 gene will be analysed by using the ANOVA test.

Results

A total of 32 samples were collected after RDT and microscopy diagnosis. After 18S PCR assays four mixed infections samples were discarded and remaining 28 mono-infection samples were analysed further (Table 1). The parasitaemia varied amongst the samples and collected samples were put to culture in the laboratory for *in vitro* adaptation. Out of the 28 confirmed samples of *P. falciparum*, 25 samples successfully adapted in culture with varying intensity but three samples with scanty parasitaemia did not grow at all in culture (Table 1). Few samples immediately adapted though few of the isolates took more than four weeks to adapt in culture. The average duration of adapting field isolates in the culture varied between 2–3 weeks.

The drug sensitivity assay was performed on all the 25 adapted isolates using the SMI assay. The trend *in vitro* responses of the tested field isolates to the drugs are represented in Figure 2. The isolates tested for AS revealed sensitivity to the drug though the range of IC₅₀ 2.26–4.08 nM indicated the rise of IC₅₀ values in field isolates as reported earlier. The mean IC₅₀ values for these drugs have been listed (Table 2). The CQ resistant isolates showed schizont maturation at 8 pmols or more and for AS no resistance was seen in isolates. High IC₅₀ values were observed for SD and PYR drugs clearly indicating the rise in IC₅₀ values close to their threshold resistance values. The IC₅₀ values for CQ have shown a remarkable reduction on comparison with the previous available data, whereas a slight increase in the IC₅₀ values for

Table 2. In vitro susceptibility pattern of field isolates to drugs.

Drug	No. of isolates	Drug concentration	Resistance (%)	IC ₅₀ (nM)	Resistance threshold (nM)
CQ	25	1–64 pmol/well	44	57.82	>100
AS	25	0.15–150 pmol/well	0	3.17	>4.1
SD	25	10–10,000 pmol/well	12	59.93	>300
PYR	25	0.125–1250 pmol/well	4	2.14	>4

Figure 3. In vitro IC₅₀ pattern for the tested drugs shows the measurements in the field isolates (the different colour dots in the figure represent the different field isolates of *P. falciparum*).**Table 3.** Comparison of *msp1*, *msp2* and *Pfg377* gene results (*Heterozygosity).

Gene	No. of samples analysed	Total no. of alleles	Mean MOI	He*
<i>msp1</i>	25	31	1.24	0.537
<i>msp2</i>	24	31	1.29	0.664
<i>Pfg377</i>	26	35	1.34	0.705

Figure 4. Allele typing of *Pfg377* gene - Lane 1–12 shows field samples showing 280–380 bp for *Pfg377* gene after PCR assay. Lane 13 shows 100 bp DNA ladder.

AS and IC₅₀ high values for SD and PYR were observed (Figure 3). Two isolates which were resistant to CQ (MWT 16 and 17) also had high IC₅₀ for AS values indicating an increase in the tolerance value for AS drug. MWT-14 and MWT-20 were found to have high IC₅₀ values for all the three drugs tested though MWT-14 was a single clone and MWT-20 a multiclonal on *msp1* and *msp2* gene analysis. The threshold IC₅₀ for in vitro resistance to CQ has been estimated to be > 100 nM, for AS > 4.1 nM, SD > 300 nM and PYR > 4.0 nM (WHO 2001) [17]. There were IC₅₀ fluctuations in field isolates for CQ which were repeated until no shift in the values were further observed but this fluctuation was not seen for the other three drugs.

The Bonferroni multiple comparison test was used for comparing IC₅₀ values in isolates for different drugs. This test showed that for CQ the samples had significant differences whereas for AS it was the least. The test was not significant for SD, PYR though a slight rise in their IC₅₀ values were observed in our study. One way analysis of variance showed statistically significant difference (<0.0001) in IC₅₀ for the samples MWT-15 and 16 for all the drugs.

In the isolates 48.5% of the isolates studied were multiclonal in nature with two or more alleles present in *msp1* and *msp2* genes. For both the genes several multiple alleles were seen in the same ratio (22.2% in each *msp1* and *msp2*) but in different isolates revealing different genotypic variation in these genes. There was only one isolate – MWT-10, positive for both allelic families of *msp1* and *msp2*. Among the parasite population 16 distinct haplotype patterns were observed and the prominent genotype pattern was MAD20 (70.3%) for *msp1* and FC27 (74.0%) for *msp2*. The parameters for the genetic diversity are listed in Table 3.

Pfg377 alleles were examined in the cryopreserved and the adapted samples simultaneously as described previously [22]. The allele typing of *Pfg377* gene in the 26 isolates revealed four different genotypes viz Type A, B, C and D varying in sizes, between 280 and 380 bp with predominant allele being 350 bp (Figure 4). The difference between the two alleles was 21 bp or multiple of 21 bps seen also in amino acid repeats in the region 3 of *Pfg377*. Type A was predominant and consisted of three different kinds of repeats DHHI, DHH and NHHI sequences repeating 4, 8 and 6 times respectively with only one kind of deletion (HN—DH) (Table 4). Type B was the second most prevalent genotype with repeats similar to that of type A but with variation in the number of repeats and no deletions was observed (Table 4). Type C consisted of only one adapted isolate [MWT-7] with variation in the number of repeats along with two types of deletions (N-Q 42 bp and D-H 21 bp). Type D consisted of also only one adapted isolate (MWT-12) deletions of different types varying in number of repeats (Table 4). Type A was seen in 16 samples out of the total 26 isolates in cryopreserved and adapted samples of the same isolate. The cryopreserved and adapted samples of MWT-4, MWT-7, MWT-8, MWT-12 and MWT-17 showed difference in their genotypes on allele typing indicating the presence of multiple clones in the same sample on sequence comparisons (Table 4). The repeats seen in these samples were of three kinds though their number of repeats varied in different isolates. Four types of deletions viz HNHIDH/DHHIDHH/

Table 4. Sequencing results for *Pfg377* gene in *P. falciparum* isolates of Mewat.

S. No.	Sample No.	Repeats				Deletion			
		DHHI	DHH	NHHI	No deletion	HNHHIDH 21 bp	NHHIDHHN- HHIDHQ 42 bp	HNHHIDHHN- HHIDH 42 bp	DHHIDHH 21 bp
1.	MWT-1	4	8	6		✓			
2.	MWT-1*	4	8	6		✓			
3.	MWT-2	4	8	6		✓			
4.	MWT-2*	4	8	6		✓			
5.	MWT-3	4	8	6		✓			
6.	MWT-3*	4	8	6		✓			
7.	MWT-4	4	8	6		✓			
8.	MWT-4*	4	9	7	✓				
9.	MWT-6	4	9	7	✓				
10.	MWT-6*	4	9	7	✓				
11.	MWT-7	4	9	7	✓				
12.	MWT-7*	3	7	5			✓		✓
13.	MWT-8	4	8	6		✓			
14.	MWT-8*	4	9	7	✓				
15.	MWT-9	4	8	6		✓			
16.	MWT-9*	4	8	6		✓			
17.	MWT-10	4	8	6		✓			
18.	MWT-10*	4	8	6		✓			
19.	MWT-11	4	8	6		✓			
20.	MWT-11*	4	8	6		✓			
21.	MWT-12	4	8	6		✓			
22.	MWT-12*	3	6	5				✓	✓
23.	MWT-13	4	8	6		✓			
24.	MWT-13*	4	8	6		✓			
25.	MWT-14	4	9	7	✓				
26.	MWT-14*	4	9	7	✓				
27.	MWT-16	4	8	6		✓			
28.	MWT-16*	4	8	6		✓			
29.	MWT-17	4	8	6		✓			
30.	MWT-17*	4	9	7	✓				
31.	MWT-18	4	8	6		✓			
32.	MWT-18*	4	8	6		✓			
33.	MWT-20	4	8	6		✓			
34.	MWT-20*	4	8	6		✓			
35.	MWT-21	4	8	6		✓			
36.	MWT-21*	4	8	6		✓			
37.	MWT-22	4	8	6		✓			
38.	MWT-22*	4	8	6		✓			
39.	MWT-24	4	8	6		✓			
40.	MWT-24*	4	8	6		✓			
41.	MWT-25	4	9	7	✓				
42.	MWT-25*	4	9	7	✓				
43.	MWT-26	4	9	7	✓				
44.	MWT-26*	4	9	7	✓				
45.	MWT-27	4	8	6		✓			
46.	MWT-27*	4	8	6		✓			
47.	MWT-28	4	9	7	✓				
48.	MWT-28*	4	9	7	✓				
49.	MWT-29	4	8	6		✓			
50.	MWT-29*	4	8	6		✓			
51.	MWT-30	4	8	6		✓			
52.	MWT-30*	4	8	6		✓			

*Culture adapted isolates.

NHHIDHHNHHIDHQ/HNHHIDHHNHHIDH were seen in most of the isolates, though in Type B no deletions were observed (Table 4). On sequence analysis 16 samples were found to harbor a single gametocyte haplotype. These results indicated that SNPs and nucleotide deletions were present in the field isolates. *Pfg377* was the most polymorphic loci with high expected heterozygosity (He) value when compared to *msp1* and 2 (Table 3).

The IC₅₀ values versus *Pfg377* haplotype was analysed by one way variance analysis but no correlation between the two parameters was observed. Few samples which had significant difference in the IC₅₀ did not show similar *Pfg377* profile and few others with different allele profile yet their IC₅₀ values were not different significantly. More

number of samples need to be analyzed for any existing correlation between the two parameters.

Discussion

In vitro drug sensitivity remains an important tool to assess the efficacy of the antimalarial drugs. The drugs majorly used in the ACT and the previously used CQ have been analysed in this study. Though the majority of isolates were found to be resistant to CQ, the IC₅₀ observed value for CQ was drastically low as seen in other reports [26,27]. This low value is indicative of the fact that the absence of CQ in malaria treatment has released the drug pressure with a possibility of moving towards being CQ

sensitive in future. There have been similar reports from other parts of the world [28]. In India CQ was replaced with ACT in the year 2005 and more than a decade has passed since the usage of CQ probably resulting in decline of the CQ resistant strains [26]. AS a derivative of artemisinin was used in our study as dihydroartemesinin is found unstable in drug plates coated with it [29,30]. In our study, the isolates were AS sensitive but their high IC_{50} value indicates of field isolates turning tolerant to higher doses of artesunate. Due to constant exposure to AS the increase in the drug pressure is likely to be responsible for increase in the IC_{50} values and another plausible explanation also could be the indiscriminate use of the drug resulting in slow rise of low level resistance in the parasite population [31]. was also observed There is very limited information about the emerging AS/SD/PYR resistance currently in the Indian field isolates and the increase in IC_{50} values for SD and PYR reported here indicates the parasite may be moving towards high drug levels as was observed earlier for CQ [30–32]. The possibility of selection of resistant parasite for the above three drugs are yet to be established and the comparison of gametocytes genotypes of in the same infection/ clones in the same infection is not much studied either.

We allele typed *Pfg377* the sexual stage specific gene in the field isolates which revealed several allele types present in them. By conventional PCR only the predominant genotype was detected in the PCR results and presence of more than one clone in a single infection were observed on sequence analysis. This shows that this gene can be effectively used for detecting the presence of gametocyte producing multiclonal in the field isolates. The presence of different genotypes/clones in the same infection is an important source of transmission [33]. The large number of repeats found in this gene is responsible for the high variability seen in parasites thus allowing several parasite lines to merge and survive simultaneously leading to transmission dynamics [34]. It is understood that presence of mixed gametocyte genotypes in the same sample is responsible for harbouring gametocytes for a significantly longer period of time on comparison with a single genotype where it was cleared three times faster than multiple clones [35,36]. Natural infections of *P. falciparum* seem to harbor multiple genotypes in the same host which could be due to the transmission burden in endemic regions but still, this knowledge does not improve the understanding of existing multiplicity in parasite biology [12].

The use of antimalarial drugs leads to the induction of gametocytogenesis in the parasite population though to correlate the drug resistant parasites with gametocytogenesis phenomenon has still not been substantiated [37,38]. The association between clone multiplicity, ability to produce gametocytes and longevity of *P. falciparum* infection is reported earlier and the parasite heterogeneity is beneficial for the evolution of the

parasite [39]. The *msp1* and 2 highly polymorphic genes were used to assess the multiclonal in the samples analysed as multiclonality induce greater antigenic variation than single clone infections [20]. Both the genes were found to be efficient in distinguishing the clones in analysed field isolates serving adequately to detect multiclonal [40]. Similar analysis of other gametocyte specific genes would allow inferring the gametocyte producing clones in parasite population to provide additional information for development of a diagnostic assay. The knowledge of the host dynamics with its epidemiological and evolutionary consequences is important for intervention measures to be successful and efforts should also be made to prevent transmission of *P. falciparum*.

Conclusions

The purpose of the present study was to assess the current drug sensitivity profile in the field isolates and type the *Pfg377* genes. The decrease in the IC_{50} values of CQ and increase in the AS IC_{50} values after the implementation of the ACT therapy is evident by the *in vitro* analysis of the 25 field isolates in Mewat region. The *Pfg377* allele typing in the 26 field isolates revealed different types of haplotypes [A, B, C and D] in them. The *Pfg377* allele typing is suitable for detecting carriers of low number of gametocytes which play a critical role in malaria transmission. Our study revealed different drug sensitivity profiles of field isolates from Mewat region and by allele typing of *Pfg377* gene it was found that different haplotypes of field isolates were circulating in parasite population. Active surveillance and continued monitoring of the ACT is required for identification of emerging drug resistance in the country for malaria control programmes.

Acknowledgments

We are thankful to the Indian Council of Medical Research [ICMR] for providing us with necessary grants to carry out the research work (Grant: 5/8/-7[247]V-2012-ECD-II). This paper bears the NIMR publication screening committee approval no. 037/2016.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Indian Council of Medical Research [grant number 5/8/-7[247]V-2012-ECD-II].

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