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Distribution of Mutations Associated with Antifolate and Chloroquine Resistance among Imported *Plasmodium vivax* in the State of Qatar

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Abstract. *Plasmodium vivax* is the most prevalent parasite worldwide, escalating by spread of drug resistance. Currently, in Qatar, chloroquine (CQ) plus primaquine are recommended for the treatment of *P. vivax* malaria. The present study examined the prevalence of mutations in dihydrofolate reductase (*dhfr*), dihydropteroate synthase (*dhps*) genes and CQ resistance transporter (*crt-o*) genes, associated with sulphadoxine-pyrimethamine (SP) and chloroquine resistance, among imported *P. vivax* cases in Qatar. Blood samples were collected from patients positive for *P. vivax* and seeking medical treatment at Hamad General Hospital, Doha, during 2013–2016. The Sanger sequencing method was performed to examine the single nucleotide polymorphisms in *Pvdhfr*, *Pvdhps*, and *Pvcrt-o* genes. Of 314 examined *P. vivax* isolates, 247 (78.7%), 294 (93.6%) and 261 (83.1%) were successfully amplified and sequenced for *Pvdhfr*, *Pvdhps*, and *Pvcrt-o*, respectively. Overall, 53.8% (N = 133) carried mutant alleles (58R/117N) in *Pvdhfr*, whereas 77.2% (N = 227) and 90% (N = 235) isolates possessed wild type allele in *Pvdhps* and *Pvcrt-o* genes, respectively. In addition, a total of eleven distinct haplotypes were detected in *Pvdhfr*/*Pvdhps* genes. Interestingly, K10 insertion in the *Pvcrt-o* gene was observed only in patients originating from the Indian subcontinent. The results suggested that CQ remains an acceptable treatment regimen but further clinical data are required to assess the effectiveness of CQ and SP in Qatar to support the current national treatment guidelines. In addition, limited distribution of genetic polymorphisms associated with CQ and SP resistance observed in imported *P. vivax* infections, necessitates regular monitoring of drug resistant *P. vivax* malaria in Qatar.

INTRODUCTION

Malaria continues to be a major health problem in Southeast Asia and Africa.[1] Globally, approximately 8% of estimated cases are due to *Plasmodium vivax* in Africa, but it increases to 47% in Southeast Asia. In the recent years, a trend of increasing malaria prevalence and malaria morbidity in South and Southeast Asian region has also been witnessed. However, it is not entirely clear at this point if this trend is the result of failure of interventions or if other causes, such as changing environments and population movements are important cofactors.[1,2] Furthermore, until recently, *P. vivax* malaria has received less attention than *Plasmodium falciparum* malaria. However, in recent years, several studies have documented the contribution of *P. vivax* to severe malaria including multiple organ dysfunctions in the Indian subcontinent.[3,4]

Qatar is situated in the Arabian Peninsula, with a total population of 2.6 million (2016 census according to the Ministry of Development Planning and Statistics, Qatar) and more than 88% of these are expatriates from the Indian subcontinent, North and East Africa, Southeast Asia and Middle East. Indigenous malaria transmission has been eliminated from Qatar but the risk of imported malaria still exists because of the large number of immigrant workers originating from the Indian subcontinent and Africa.[5,6] The incidence of malaria, mostly due to *P. vivax*, has increased over the past decade in Qatar and the majority of cases seen in the period between August and October, which confirms the importation of infection by expatriates from their respective countries on their return from summer vacation.[6,7]

Antimalarial drug resistance is the most persistent problem confronting malaria control programs in many endemic countries.[1,8] Moreover, the spread of chloroquine (CQ) and sulphadoxine–pyrimethamine (SP) drug resistance in *P. vivax* is becoming more widespread and has a significant public health implication in the control program.[9–11] Currently, CQ followed by primaquine is the first-line regimen for the treatment of *P. vivax* malaria in Qatar.

Fansidar (SP), an antifolate drug combination, is one of the extensively used antimalarial drugs, after CQ resistance rise in *P. falciparum,* throughout the world because of its low cost and relative safety.[12] SP has never been recommended for treatment of *P. vivax* malaria in Qatar; however, the coexistence of *P. falciparum* and *P. vivax* and misdiagnosis of *Plasmodium* species suggest that unnecessary treatment of *P. vivax* malaria with SP may be inadvertently led to the simultaneous selection of SP resistant *P. vivax* by inducing drug pressure on *P. vivax* isolates. Furthermore, in countries where the SP has been used intensively for *P. falciparum* malaria, resistance has also been appearing in *P. vivax* populations.[13–18]

Molecular epidemiology studies have suggested that *P. vivax* resistance to SP is associated with point mutations in *Pvdhfr* and *Pvdhps* genes encoding dihydrofolate reductase

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(DHFR) and dihydropteroate synthase (DHPS), major proteins involved in the folate biosynthesis pathways.[13,14] Four nonsynonymous mutations of *Pvdhps* at positions S382F/A, A437G, K540E, and A581G have been implicated, these are equivalent to known nonsynonymous mutations in *P. falciparum dhps* at positions S436F/A, A437G, K540E, and A581G, respectively.[19–21] Among these mutations, the A383G single mutation, A383G A553G double mutation, and S383A A553G triple mutation were reported in many endemic countries.[18] With regard to *Pvdhfr*, twenty nonsynonymous mutations have been described.[14,17] Of these, mutations at codons 57, 58, 61, 117, and 173 described to be involved in antifolate resistance,[13,22] which corresponded to 51, 59, 108, and 164 in *P. falciparum*.[22] Similarly, five mutations in the *Pvdhps* at codon 382, 383, 512, 553, and 585 have also been suspected to be involved in SP resistance.[19–22] However, it has been speculated that there may be a known innate resistance of *P. vivax* to sulphadoxine because of the steric hindrance caused by the V585 in *Pvdhps* to the binding site of sulphadoxine.[23]

The drug resistance to CQ in *P. vivax* has been reported from different malaria endemic regions.[9] But, unlike in *P. falciparum*, the molecular mechanisms behind CQ resistance in *P. vivax* remain unclear. Nevertheless, mutations in K10 insertion in the *Pvcrt-o* gene have been identified as a possible molecular marker of CQ resistance in *P. vivax*.[24] In addition, recently, a CQ mutant allele of *P. vivax* (*Pvcrt-o*) has shown an association with in vivo and in vitro drug susceptibility in malaria-endemic countries.[25]

With the increasing reports of severe manifestations because of *P. vivax* in South Asia region,[2] and the rampant use of CQ and SP for the treatment of malaria in these countries, there is a need for monitoring antimalarial drug efficacy and drug resistance in Qatar for prompt management of imported *P. vivax* malaria as most of the immigrant workers come from the Indian subcontinent.[5–7,26] Therefore, this study aimed to determine the distribution of mutant *Pvdhfr*, *Pvdhps*, and *Pvcrt-o* genes involved in resistance to different antimalarial drugs in *P. vivax*. The pattern of mutations present in this study will provide valuable molecular information on antifolate and CQ drug resistance, and that may be useful for epidemiological mapping of drug-resistant *P. vivax* malaria in Qatar.

MATERIALS AND METHODS

Study site, ethics, and sample collection. The study was conducted in Doha, Qatar (25.3548° N and 51.1839° E). This study received ethical approval from the Institutional Review Board of WCM-Q and HMC (Protocol no. 14-00097). During January 2013–September 2016, on a written informed consent from adult and parents of minors, a total of 314 *P. vivax* malaria cases from 12 countries were enrolled in this study (Figure 1). Blood samples were collected from the patients with uncomplicated malaria (N = 314) attending the Hamad General Hospital, a premier nonprofit health care provider in Doha, Qatar.

All of the samples were screened for the *Plasmodium* infection by light microscopic examination of Giemsa-stained blood smears and subsequently confirmed by nested polymerase chain reaction (PCR).[27] The patient's sociodemographics and clinical parameters such as age, nationality, clinical history, parasitaemia, and hemoglobin were collated from a patient's medical records. In addition, information on risk factors such as travel history and blood transfusion was also recorded on structured questionnaire.

DNA extraction and amplification of *Pvdhfr*, *Pvdhps*, and *Pvcrt-o*. Genomic DNA was isolated using the QIAamp DNA blood mini kit following the manufacturer's instructions

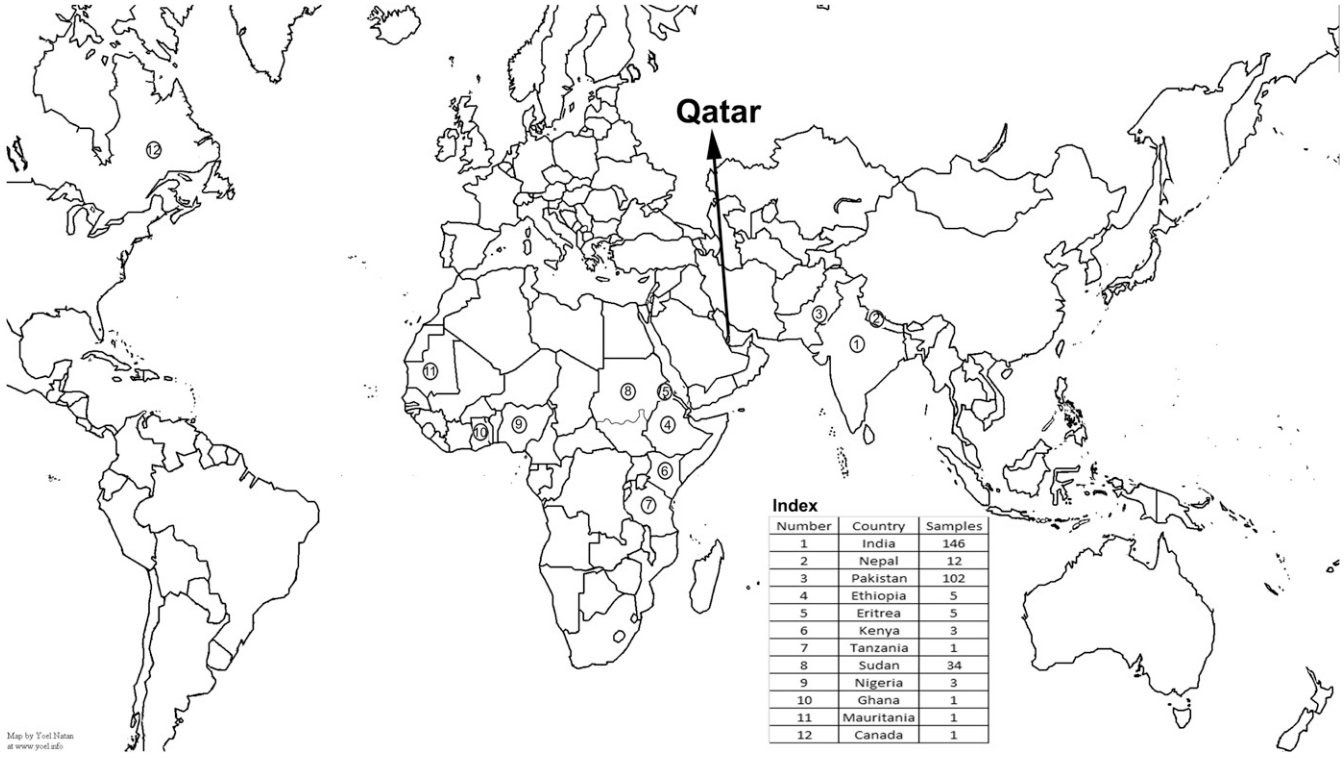

FIGURE 1. A map showing the countries of origin of the patients from whom *Plasmodium vivax* samples were collected in Doha, Qatar.

(Qiagen, Valencia, CA). Target sequences of the *Pvdhfr, Pvdhps*, and *Pvcrt-o* genes harboring putative mutations associated with SP and CQ resistance were amplified and sequenced. The outer and nested primers and thermal cycling conditions adapted as previously described[16,24,28] and are summarized in Table 1. The expected band sizes for *Pvdhfr*, *Pvdhps*, and *Pvcrt* were 785, 703, and 1194 bp, respectively.

Sequencing and data analysis. Amplified secondary PCR products of *Pvdhfr*, *Pvdhps*, and *Pvcrt* genes were purified using the QIAquick PCR Purification Kit according to manufacturer's instructions (Qiagen). The sequencing reaction for all three genes was performed in the forward and reverse directions using respected nested PCR primers. Sanger sequencing was performed (Genewiz Inc., South Plainfield, NJ) and sequence analysis was performed using Leaser gene (SeqMan™ Version 7.0; DNASTAR, Madison, WI) and Geneious version R10 (www.geneious.com). Sequences of a *P. vivax crt-o* isolate (GenBank: EU333972.1), PPPK-dhps isolate (GenBank: EU478871.1), and dhfr-TS isolate (GenBank: EU478858.1) were used as references for studying polymorphisms in *Pvcrt*, *Pvdhps*, and *Pvdhfr*, respectively.

Statistical analysis. A descriptive analysis was performed and estimates of prevalence along with their 95% confidence intervals (CI) were obtained (Table 2).

RESULTS

Demographic characteristics of the study population. A total of 314 blood samples were collected from patients with microscopically confirmed *P. vivax* in HMC hospital, Doha. The age range was 3–73 years with a mean age of 32.1 years (SD = 11.9). The majority of patients were from India (46.5%, *N* = 146) with the rest belonging to Pakistan (32.5%, *N* = 102), Sudan (10.8%, *N* = 34), Africa (6%, *N* = 19), Nepal (3.8%, *N* = 12) and Canada (*N* = 1, 0.3%) who had a travel history of India (Figure 1). All patients had a history of travel to their country of origin ranged between 1 day and 13 months, but none of them had blood transfusion history.

Mutations and haplotypes in *P. vivax dhfr*, *dhps*, and *crt-o* gene. For *Pvdhfr*, *Pvdhps*, and *Pvcrt-o*, 247 (78.7%), 294 (93.6%), and 261 (83.1%) *P. vivax* isolates, respectively were successfully amplified and sequenced.

With regard to *Pvdhfr*, 46.2% (*N* = 114) contained the wild-type *Pvdhfr* genotype ANSSI (codons 15, 50, 58, 117, and 173) and 53.8% (*N* = 133) of samples possessed mutation at codons (58R and 117N) (Table 2). Of 53.8%, *Pvdhfr* double mutation, 58R**/**117N, was observed in 40.5% (*N* = 100) cases of *P. vivax* infection (95% CI: 34.31–46.89%) with 10.9% (*N* = 27) single mutant at codon 117**N** (95% CI: 7.33–15.50%) followed by 2.4% (*N* = 6) at codon 58R (95% CI: 0.90–5.21%) but no case exhibited mutation at codon 15, 50, and 173 of *Pvdhfr* (Table 2). The haplotype analysis of *Pvdhfr* revealed four distinct allelic forms from all samples except for samples originating from Nepal, where only two haplotype (wild type and double mutant) was recorded (Table 2).

With respect to the *Pvdhps* gene, 294 (92.4%) samples were successfully sequenced and the majority (77.1%, *N* = 227) of the isolates had a wild type *Pvdhps* allele SAKAV (codons 382, 383, 512, 553, and 585). Of the remaining isolates, 10.2% (*N* = 30) harbored single mutation at codon 383G (95% CI: 6.99–14.25%) and the frequency distribution of double mutant alleles (383G/553G) was 12.6% (*N* = 37) (95% CI: 9.02–16.93%) (Table 2). Furthermore, we detected three haplotypes in our populations and single mutant haplotype (383**G**) as well as double mutant haplotype (383G/553G) found from all over the country (Table 2).

In addition, 11 different haplotypes were found, when we grouped *Pvdhfr* and *Pvdhps* genes. The majority of the isolates showed the wild type (40.0%) followed by double mutant alleles (Figure 2). Triple (8.0%) and quadruple (10.0%) mutations were also detected in this study, especially among samples collected from patients of Indian origin (Table 3).

The *Pvcrt-o* gene was successfully sequenced in 251 (78.9%) isolates. The majority of isolates (90.0%, *N* = 235) carried wild type, without K10 insertion (95% CI: 86.22–95.52%) (Table 2). Of note, synonymous mutations or K10 insertions

TABLE 1

Primer sequence and PCR conditions used for amplification of *Plasmodium vivax* drug resistance genes

Genes		Primers	Sequence	PCR conditions	PCR amplified product (bp)	References
Pvdhfr	PF	Outer	5′-accgcaccagttgattcctac-3′	94°C × 10 minutes, 35 cycles of [94°C × 30 seconds, 58°C × 60 seconds, 72°C × 60 seconds], 72°C × 10 minutes	1,200	16
	PR		5′-tgttaaagctgaagtacacgag-3′			
	NF	Nested	5′-atggaggacctttcagatgt-3′	94°C × 10 minutes, 30 cycles of [94°C × 30 seconds, 54°C × 60 seconds, 72°C × 60 seconds], 72°C × 10 minutes	785	
	NR		5′-aacgcattgcagttctccga-3′			
	NF	Sequencing	5′-atggaggacctttcagatgt-3′	Purified and sequenced using Sanger method	–	
	NR		5′-aacgcattgcagttctccga-3′			
Pvdhps	PF	Outer	5′-attccagagtataagcacagcacatttgag-3′	94°C × 10 minutes, 35 cycles of [94°C × 30 seconds, 58°C × 60 seconds, 72°C × 60 seconds], 72°C × 10 minutes	1,463	28
	PR		5′-ctaaggttgatgtatccttgtgagcacatc-3′			
	NF	Nested	5′-aatggcaagtgatggggcgagcgtgattga-3′	94°C × 10 minutes, 30 cycles of [94°C × 30 seconds, 58°C × 60 seconds, 72°C × 60 seconds], 72°C × 10 minutes	703	
	NR		5′-cagtctgcactccccgatggccgcgccacc-3′			
	NF	Sequencing	5′-aatggcaagtgatggggcgagcgtgattga-3′	Purified and sequenced using Sanger method	–	
	NR		5′-cagtctgcactccccgatggccgcgccacc-3′			
Pvcrt	PF	Outer	5′-cgctgtcgaagagcc-3′	94°C × 10 minutes, 35 cycles of [94°C × 60 seconds, 54°C × 60 seconds, 72°C × 60 seconds], 72°C × 10 minutes	1,194	24
	PR		5′-agtttccctctacacccg-3′			
	NF	Sequencing	5′-cgctgtcgaagagcc-3′	Purified and sequenced using Sanger method	–	
	NR		5′-agtttccctctacacccg-3′			

NF = nested forward; NR = nested reverse; PCR = polymerase chain reaction; PF = primary forward; PR = primary reverse.

TABLE 2
Mutations analysis of *Pvcrt*, *Pvdhfr*, and *Pvdhps* genes in study populations

Gene	Mutation type	India		Nepal		Pakistan		Sudan		Rest of Africa		Total	
		N (%)	95% CI	*N* (%)	95% CI	*N* (%)	95% CI	*N* (%)	95% CI	*N* (%)	95% CI	*N* (%)	95% CI
Pvdhfr	117N	3 (2.52)	(0.52–7.13)	–	–	22 (27.5)	(18.10–38.62)	1 (3.33)	(0.08–17.21)	1 (9.09)	(0.23–41.28)	27 (10.93)	(7.33–15.50)
	58R	1 (0.84)	(0.02–4.56)	–	–	2 (2.5)	(0.30–8.74)	3 (10)	(2.11–26.53)	–	–	6 (2.43)	(0.90–5.21)
	58R, 117N	59 (49.58)	(39.93–58.45)	4 (57.14)	(18.41–90.10)	21 (26.25)	(17.04–37.29)	9 (30)	(14.73–49.40)	7 (63.64)	(30.79–89.07)	100 (40.49)	(34.31–46.89)
	Wild	56 (47.06)	(37.51–56.00)	3 (42.86)	(9.90–81.59)	35 (43.75)	(32.67–55.30)	17 (56.67)	(37.43–74.54)	3 (27.27)	(6.02–60.97)	114 (46.15)	(39.81–52.59)
Pvdhps	383G	10 (7.41)	(3.61–13.20)	2 (18.18)	(2.28–51.78)	9 (9.28)	(4.33–16.88)	7 (22.58)	(9.59–41.10)	2 (10.00)	(1.23–31.70	30 (10.20)	(6.99–14.25)
	383G, 553G	28 (20.74)	(14.25–28.56)	3 (27.27)	(6.02–60.97)	2 (2.06)	(0.25–7.25)	2 (6.45)	(0.79–21.42)	2 (10.00)	(1.23–31.70	37 (12.59)	(9.02–16.93)
	Wild	97 (71.85)	(63.47–79.25)	6 (54.55)	(23.38–83.25)	86 (88.66)	(80.61–94.20)	22 (70.97)	(51.96–85.78)	16 (80.00)	(56.34–94.27)	227 (77.21)	(71.98–81.88)
Pvcrt	Mutant	11 (8.33)	(4.23–14.42)	1 (10.00)	(0.25–44.50)	13 (16.46)	(9.06–26.50)	1 (3.45)	(0.09–17.76)	–	–	26 (9.96)	(6.61–14.25)
	Wild	121 (91.67)	(85.58–95.77)	9 (90.00)	(55.50–99.74)	66 (83.54)	(73.51–90.94)	28 (96.55)	(82.24–99.91)	11 (100.00)	–	235 (90.04)	(85.75–93.39)

CI = confidence interval; N = number.

(AAG) were found in 11.3% (*N* = 24/212) of isolates from India and Pakistan (95% CI: 6.48–13.78%) and was absent in *P. vivax* parasites from Africa, except one (3.45%) patient from Sudan.

DISCUSSION

The molecular markers associated to antimalarial drug resistance are valuable tools for the surveillance of resistant malarial parasites. They are useful to anticipate risks associated with the emergence, the spread of antimalarial parasite resistance, and to alert policy makers. The associations of point mutations in the *Pvdhfr* and *Pvdhps* genes have been well established with antifolate drug resistance.[29,30] A number of in vitro and in vivo studies have also suggested that these molecular markers provide valuable information about the trends of SP resistance in *P. vivax*.[15,31,32] Furthermore, in *Pvdhfr* gene, it has been well established that mutations at S58R and S117N arise first and increases resistance more than wild type under drug pressure.[13–15,33]

In the present study, high prevalence (54%) of both *Pvdhfr* 58R and 117N mutation was observed. However, mutations at codons I13L, P33L, F57L, T61M, I172V, and I173L, which have been reported earlier from different regions,[16,34–38] were not found in our studied population. Furthermore, delayed parasite clearance and treatment failure of SP has been associated with double (N117/R58), triple (N117/R58/L57), and quadruple mutations (L57/R58/M61/T11).[13,15,22,34–38] In this study, among the mutants identified, the *Pvdhfr* double mutant at positions 58R and 117N were predominant (40%), suggesting the drug pressure on these parasites. Our observation corroborates the previous reports from Pakistan, Nepal, and Sudan.[39–41] However, no triple/quadruple mutations were detected, which is in contrast with previous findings from India[16,36] as India has different degrees of malaria endemicity, and the levels of mutations from the different geographical state is also varied.[42]

With regard to the *Pvdhps* gene, majority of our isolates (77.2%) carried wild type alleles and frequency of single mutant (383G) and double mutant genotypes (383G/553G) was 10.2% and 12.6%, respectively, which have been found to be directly related to sulphadoxine resistance[43] and associated with reduced sensitivity to both sulfa drugs and sulfones.[23,28] In addition, the association of SP treatment failure has also been reported with *Pvdhps* 553G/383G in combination with *Pvdhfr* double mutation 58R/117N and increasing prevalence of these mutations may further reduce the sensitivity to antifolate drugs in *P. vivax* population.[44,45] The multiple mutation pattern (combination of 58, 117 + 383 triple mutant) was found from all over the country, although it was in low level. However, the other triple (117 + 383, 553) and quadruple (58, 117 + 383, 553) combinations were observed mainly in the patients originating from India. Such multiple mutations pattern was also reported previously from India.[34]

The association of molecular markers i.e., *Pfcrt* and *Pfmdr1* with the CQ drug resistance in *P. falciparum* malaria has been well documented; however, little is known about the possible mechanisms of CQ resistance (*Pvmdr1* and *Pvcrt-o* genes) in *P. vivax* malaria.[46,47] In the present study, wild type allele was found to be predominant and K10 insertions (AAG) was found in 11.2% of the Indian subcontinent samples, suggesting

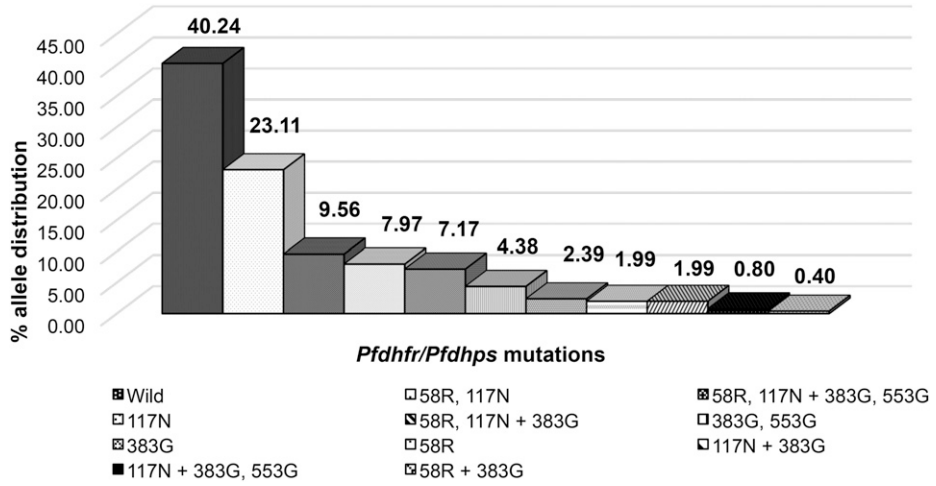

FIGURE 2. Prevalence of grouped *Pvdhfr/Pvdhps* mutant alleles in *Plasmodium vivax* linked to sulphadoxine-pyrimethamine treatment failure.

commencement of a trend toward decreased CQ sensitivity in these countries.

Overall, in the present study, a large proportion of the isolates harbored wild-type genotype of *Pvdhps* and *Pvcrt-o* genes. However, *P. vivax* parasites carried the same frequency of wild-type *Pvdhfr* allele (46.5%) and double mutations at 58R/117N (53.8%), suggesting that both the alleles are still common in the Indian subcontinent and Africa and could cause structural changes in *Pvdhfr* and lead to decreased binding to pyrimethamine.[48,49] Nevertheless, it has been shown that *P. vivax* isolates with multiple mutations in *Pvdhfr* and *Pvdhps* can respond adequately to SP.[28] Thus, in the present study, the absence of triple/quadruple mutations strongly suggests that imported *P. vivax* infection in Qatar can respond adequately to antifolate drugs. However, the presence of double mutations in *Pvdhfr* and *Pvdhps* genes indicates the regular monitoring is essential for malaria treatment point of view. The limitation of the present study was that we were unable to perform the therapeutic efficacy study; hence, couldn't correlate the molecular findings with treatment outcome and in vitro drug sensitivity. Therefore, in future, a therapeutic efficacy study should be performed to correlate genotypic observations with phenotypic data.

The current guidelines for treatment of malaria at HMC include administration of chloroquine, followed by primaquine for patients with *P. vivax* malaria and coartem or quinine plus doxycycline (adults)/or clindamycine (children) for patients with *P. falciparum* malaria. In addition, those with mixed infections were usually treated as *P. falciparum* malaria.[50] In the present study, all the patients were from the Indian subcontinent and Africa, where SP alone has never been recommended for the treatment of *P. vivax* malaria but artesunate + SP combination is used for treatment of *P. falciparum* malaria in some African countries.[1] The incidence of *P. falciparum*/*P. vivax* co-infection is most common in malaria endemic countries but because of a wrong diagnosis and extensive use of SP to treat *P. falciparum* malaria patients,[16] *P. vivax* population often exposed accidentally to SP, resulting in a selection pressure which might be reflected in the observed mutation pattern in the *Pvdhfr* and *Pvdhps* genes in our parasite populations.

Plasmodium vivax may cause relapse within a few weeks to a few months after initial infection.[51] As reported indigenous malaria transmission has been eliminated from Qatar, the patients who were positive for *P. vivax* and did not have a recent travel history may likely be the cases of relapses.

In conclusion, this was the first comprehensive molecular study carried out in Qatar focusing on mutation in *Pvdhfr*, *Pvdhps*, and *Pvcrt-o* genes that were strongly associated with SP and CQ resistance. This study shows relatively low distribution of these mutations associated with antifolate and chloroquine resistance, which suggest that the *P. vivax* parasites may still be susceptible to SP and CQ and any combination of antimalarial drug with antifolates that have been recommended for *P. falciparum* might be effective in *P. vivax*

TABLE 3
Different haplotypes of *Pvdhfr* and *Pvdhps* genes from the different countries

Gene	Mutation type	India (*N* = 120)	Nepal (*N* = 9)	Pakistan (*N* = 80)	Sudan (*N* = 31)	Rest of Africa (*N* = 11)
Pvdhfr	117N	1 (0.83)	0.00	18 (22.50)	1 (3.23)	0
	58R	1 (0.83)	0.00	1 (1.25)	3 (9.68)	0
	58R, 117N	32 (26.67)	1 (11.11)	19 (23.75)	2 (6.45)	4 (36.36)
Pvdhps	383G	4 (3.33)	0.00	2 (2.50)	0.00	0.00
	383G, 553G	5 (4.17)	2 (22.22)	2 (2.50)	2 (6.45)	0.00
Pvdhfr + *Pvdhps*	117N + 383G	1 (0.83)	0.00	4 (5.00)	0.00	0.00
	58R + 383G	0.00	0.00	1 (1.25)	0.00	0.00
	117N + 383G, 553G	1 0.83	0.00	0.00	0.00	1 (9.09)
	58R, 117N + 383G	5 (4.17)	2 (22.22)	2 (2.50)	7 (22.58)	2 (18.18)
	58R, 117N + 383G, 553G	22 (18.33)	1 (11.11)	0.00	0.00	1 (9.09)
	Wild	48 (40.00)	3 (33.33)	31 (38.75)	16 (51.61)	3 (27.27)
	Total	120 (100.00)	9 (100.00)	80 (100.00)	31 (100.00)	11 (100)

N = number.

malaria also. However, the presence of high prevalence of double mutation in the *Pvdhfr* gene demonstrates a need of continuous surveillance studies. The development and spread of drug-resistant parasite strains is a major obstacle to the malaria control and elimination program. Therefore, the results of the present study provide baseline data on the extent of SP drug resistance in *P. vivax*, which might be helpful for the enrichment of molecular surveillance of antimalarial resistance and will be useful for developing and updating antimalarial guidance for imported cases in Qatar.

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