Influx of diverse, drug resistant and transmissible Plasmodium falciparum into a malaria-free setting in Gulf Cooperation (GCC) countries.

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Abstract

Background
Successful malaria control programs have interrupted local malaria transmission in almost all the Gulf Cooperation Council (GCC) countries. However, a massive influx of imported malaria via migrant workers from endemic areas sustains a threat for the re-introduction of local transmission. Here we examined the origin of imported malaria into one of the GCC countries (Qatar) and assessed the extent of genetic diversity, and carriage of drug resistance genes of imported Plasmodium falciparum and it’s potential to re-introduce the disease.

Methods
We examined imported malaria reported in Qatar, between 2013 and 2016. We focused on P. falciparum infections and estimated total parasite and gametocyte density using qPCR and qRT-PCR, respectively. In addition, we examined ten neutral microsatellites and four drug resistance genes, Pfmrp1, Pfcr, Pfmdr1 and Pfkelch13 , to assess the extent of diversity of imported P. falciparum and its potential carriage of drug resistance genotypes respectively.

Results
The majority of imported malaria comprised P. vivax , while P. falciparum and mixed species infections ( P. falciparum /P. vivax ) were less frequent. The main origin of P. vivax was the Indian subcontinent, while P. falciparum was most apparent among expatriates from Africa. Imported P. falciparum was highly diverse carrying multiple genotypes as well as early and late gametocytes. We observed a high prevalence of SNPs implicated in drug resistance among imported P. falciparum , with some novel SNPs in Pfkelch13 .

Conclusions
The high influx of genetically diverse P. falciparum, with multiple drug resistance marker gene mutations and high capacity of producing gametocytes, sustains threat for re-introduction of drug resistant malaria into GCC countries. This scenario highlights the impact of current globalisation of movement of humans in reintroducing malaria infections to areas targeted for elimination.

Background
The Gulf Cooperation Council (GCC) countries, Bahrain, Kuwait, Oman, Qatar, United Arab Emirates (UAE) and Saudi Arabia, have achieved great success in malaria control. The scale up of control efforts, established in the 1950s, has extended the interruption of local transmission, and led to malaria-free status in all of the countries, with exception of Saudi Arabia, where limited foci of indigenous malaria exist [1, 2] and sporadic outbreaks in Oman [3]. This has encouraged the health authorities of GCC countries to shift policy towards a malaria-free Arabian Peninsula [4], and to focus on strategies to prevent re-introduction, via sustainable vector control, improved surveillance and prompt case management [5].

Qatar has been free from local malaria transmission since the 1970s [6], with no reports of indigenous malaria [7]. However, the influx of migrant workers from malaria-endemic countries in the Indian subcontinent and Africa, has sustained a high number of imported cases, representing a major threat for the re-introduction of local transmission. Migrants constitute the vast proportion of GCC countries’ population, reaching > 80% in Qatar [8]. In the last two decades there has been an incremental trend in the flow of immigrant workers to Qatar, associated with an increase in the reported cases of imported malaria [7, 9, 10]. In addition to flow of imported cases, the receptivity and risk of malaria reintroduction is evident by the presence of the mosquito vectors, Anopheles stephensi and An. multicolor [11]. Similar risk factors for re-introduction of malaria prevail in other GCC countries, with high influx of expatriates from malaria-endemic areas, defying current efforts to curb local transmission in some areas. For example, repeated outbreaks of locally acquired cases, linked to imported malaria, were seen in Oman [3].

In general, the chronic nature of asymptomatic malaria infection, most commonly seen in adults, and global population movement, are major threats to effective malaria elimination [12]. Imported asymptomatic infection often carries drug resistant strains of the parasite, and the transmissible stages [9, 13], therefore pausing an imminent threat to receptive areas where transmission has been interrupted or targeted for elimination [14]. Consequently, elimination and maintaining malaria-free status is challenged by the steady stream of travelers arriving from high transmission settings.

Therefore, understanding the rates, parasite species involved, the extent of diversity and carriage of
drug resistant strains of imported malaria, would allow deployment of effective cross-border measures to limit their impact in areas targeted for elimination.

The present study examined the source of imported malaria to the transmission-free state of Qatar, and assessed the genetic complexity, drug resistance genes and ability of P. falciparum to produce gametocytes and thus transmit to the vector mosquitoes. Such knowledge would allow control programs to develop targeted policies to reduce circulating parasites, define the source of outbreaks and limit the risk of reintroduction of malaria.

Methods

Subjects

A total of 583 patients, reporting to Hamad General Hospital and Al-Khor Hospital, Doha, two main centers of Hamad Medical Corporation (HMC) for malaria treatment, were examined for malaria between January 2013 and October 2016. All cases were diagnosed using conventional microscopic examination of Giemsa-stained thick (100 fields) and thin blood (1000 RBCs) films. A total of 448 (76.8%) patients were positive for malaria parasites, and the origin of infection in each patient was traced to an endemic region outside Qatar, and therefore defined as imported malaria [15]. Genomic DNA from whole blood was isolated by QIAamp DNA blood mini kit as per the manufacturer's instructions (Qiagen, CA, USA). Species identification was confirmed using species-specific PCR as described previously [16]. Quantification of P. falciparum was carried out using qPCR of 18s rRNA [17]. Demographic information on the patient was collected using a structured questionnaire, including age, gender, nationality, history of travel and any previous treatment. Patients were given treatment as per the current guidelines for malaria treatment at HMC [11]

Microsatellites genotyping and multiplicity of infection (MOI)

A panel of ten unlinked polymorphic microsatellites of P. falciparum were genotyped as described elsewhere [18, 19]. Labelled PCR products were mixed with Gene-Scan™ 500 ROX internal size standard (Applied Biosystems, UK) for electrophoresis on an ABI 3130XL Genetic Analyzer (Applied Biosystems, UK). GeneMapper software version 4 (Applied Biosystems, UK) was used for scoring allele
sizes and quantifying peak heights for samples containing multiple alleles per locus. Multiple alleles per locus were scored if electrophoretic peaks corresponding to minor alleles were > 32% the height of the predominant allele [19].

**Detection and quantification of early and late *P. falciparum* gametocytes**

Quantitative RT-PCR (qRT-PCR) was used to detect and quantify mRNA from the early gametocyte-specific gene, *Pfpeg4* [20], and the late gametocyte-specific gene, *pfs25* [21]. Total RNA was first isolated from 100 µL of blood taken from a fingerpick, using the SV Total RNA Isolation System (Promega, UK). Quantitative reverse transcription and subsequent amplification (qRT-PCR) of cDNA was carried out using the High Capacity cDNA Reverse Transcription Kit (Thermo Fisher, UK). The RT-PCR conditions and primers used were previously described [17, 22].

**Amplon sequenc for characterisation of *P. falciparum* drug resistance loci**

SNPs in four *P. falciparum* genes, *Pfmrp1* (PF3D7_0112200), *Pfcrt* (PF3D7_0709000), *Pfmdr1* (PF3D7_0523000), and *PFK13* (PF3D7_1343700), implicated in resistance to several different antimalarial drugs, were typed by amplicon sequence as previously described [23]. The analysis combined multiplex PCR, and custom-designed sequence analysis using Miseq sequencing for high throughput SNP-profiling of drug resistance genes [23]. Seventy *P. falciparum* isolates were examined. The *PFK13 and Pfcrt*, genes were each amplified as one fragment, while the longer *Pfmdr1* and *Pfmrp1* genes were amplified as two fragments. The PCR was carried out in a volume of 25 µl, containing 1 µl (10 pmol) of primers, 0.4 µl of dNTPs (200µmol/L), 4 µl of Phusion HF buffer (5x) and 1 U of Phusion high-fidelity polymerase enzyme. The cycling temperature profile for all loci was: 98°C/30s, followed by 30 cycles of (98°C/10s, 64°C/4 min), and a final extension at 64°C/5 min. The PCR amplicons of all genes for each isolate were pooled and purified using Agencourt AMPure XP purification system and quantified using a Qubit double-stranded DNA (dsDNA) HS assay kit (Thermo Fisher Scientific). The sequencing was run on Illumina® MiSeq systems. Initially, libraries were prepared using Nextera XT kit according to the manufacturer's protocol (Illumina Nextera® XT DNA Sample Preparation Guide, 2012). Following PCR cleanup, the libraries were quantified using the Qubit dsDNA BR kit, and evaluated for fragment size using Agilent High Sensitivity DNA Kit for the 2100
Bioanalyzer Instrument (Agilent Technologies, Santa Clara, CA, USA). Each library was normalized for sequencing to 10 pM according to the manufacturer's protocol and following Illumina's (Illumina®, San Diego, CA, USA) technical note for cluster optimization (Illumina Nextera® Library Validation and Cluster Density Optimization, 2013). Sequencing reactions were carried out using the MiSeq Reagent Kit V2 for 50 cycles (MiSeq, Illumina). SNPs in all genes were called using the reference sequence of the *P. falciparum* 3D7 clone, version 3 (PlasmoDB, PF3D7 v3).

**Data Analysis**

All samples that contained gametocyte transcripts, as detectable by qRT-PCR, were then analysed for association between gametocyte carriage and total parasitemia. A Mann Whitney U test was used to examine differences between the density of mature and early gametocytes. Spearman's rank order correlation test was used to examine any association between total parasite density (18S rRNA copy number) and the density of either late gametocytes (*Pfs25* copy number) or early gametocytes (*Pfpeg4* copy number).

Microsatellite allele data were filtered to retain only minor alleles having a peak height of >33 % of the corresponding predominant alleles if more than one allele was present at any locus. Genetic diversity parameters were calculated for the entire dataset using GenAlex v6.5 [24], including polymorphism at each locus as well as overall and within sub-population diversity, between parasites originated from Africa and The Indian subcontinent. Expected heterozygosity was calculated using the formula for ‘unbiased heterozygosity’ also termed haploid genetic diversity, $H_e = \frac{n}{(n-1)}[1-\sum p^2]$ where $n$ is the number of isolates and $p$ the frequency of each different allele at a locus [25].

Population differentiation was assessed by estimating Wright’s $F_{ST}$ index using the Fstat computer package Version 2.9.3.2. Two estimators of $F_{ST}$ ($G_{ST}$ and $\theta$) [26, 27] were used to estimate genetic differentiation between imported parasites from the Indian Subcontinent and Africa.

Multiplicity of infection, defined as the presence of multiple genotypes per infection, was determined by the detection of more than one allele at a locus. To avoid over-estimation of low abundance alleles, only minor alleles having a peak height of >33 % of the corresponding predominant alleles were
accepted. The proportion of samples with more than one allele across the ten loci was used to represent the multiplicity of infection (MOI). The maximum number of alleles across the ten loci was used as an index for minimum number of clones per infection (MNC). The overall mean for the index value for each sample was then calculated.

Results

Demographic characteristics of imported malaria cases in Qatar

Out of 583 patients (all expatriates) examined for malaria, between January 2013 and October 2016 in Hamad General Hospital and Al-Khor Hospital, 448 (76.8%) tested positive: 318 *P. vivax* (70.9%), 118 *P. falciparum* (26.3%) and 12 (2.7%) mixed *P. vivax*/*P. falciparum* infection (Table 1). The vast majority of the malaria-positive patients were males, *P. vivax* (94.3%); *P. falciparum* (84%), with a mean age of 32 yrs and 33 yrs, respectively, reflecting the fact that the predominate attending patients were young male.

The major origin of those presenting with *P. vivax* was the Indian Subcontinent (83%\%, n = 264): India (46%, n = 146), Pakistan (32.1%, n = 102) and Nepal (3.8%, n = 12). A smaller proportion of the *P. vivax* cases were from Africa (16%, n = 53) (Table 1).

Unlike *P. vivax*, the main origin of *P. falciparum* infection was Africa (East African [76.1%, n = 67] West & Central African countries [23.9%, n = 21], followed by the Indian Subcontinent (20.3%, n = 24) and other countries (5.1%, n = 6) (Table 1).

Parasitaemia and gametocytaemia among imported malaria cases

Ninety of the 118 *P. falciparum* infections were further examined for total parasite and gametocyte density, using qPCR and qRT-PCR, respectively, and diversity of 10 microsatellites and alleles of four genes linked to drug resistance.

The total *P. falciparum* density among imported cases ranged widely between 32 and 9,218,498 parasites/ml blood with a median 82,783 parasites/ml. The median parasite density among imported cases from the Indian Subcontinent (99,572 parasites/ml) was not significantly higher than that from Africa (88,504 parasites/ml) (p=0.394).

Seventy-three *P. falciparum* isolates were examined by qRT-PCR to detect and quantify transcripts of
genes expressed in early (Pfpeg4) and late gametocytes (Pfs25). The prevalence of all gametocytes was 74% (n=54); with 9.6% (n=7), 37% (n=27) and 27.4% (n=20) of patients carrying only early stages, only late stages or a mixture of both stages, respectively.

Early gametocytes were found at a relatively lower density, ranging between 14 and 3781/ml blood, with a median of 1011 early gametocytes/ml blood, compared to late gametocytes, which presented at a higher density overall (Mann Whitney U test, p = 0.003, range 16 - 15289 gametocytes/ml blood, median 136 gametocytes/ml blood). No correlation was found between total parasitaemia (18S rRNA copy numbers) and either late gametocytes (Pfs25 copy number) ($r_s = 0.008$, $p = 0.946$) or early gametocytes (Pfpeg4 copy number) ($r_s = 0.031$, $p = 0.835$) (Figure1) and early gametocyte density ($p = 0.857$).

Genetic diversity and structure of imported P. falciparum parasites

Microsatellite polymorphism

All the examined microsatellites were highly polymorphic among P. falciparum isolates originating from both Africa and the Indian Subcontinent (Table 2). The number of alleles per locus was higher among the African isolates, ranging from 5 for pfg377 to 18 for polyα, compared to the Indian Subcontinent isolates, ranging from 3 for 2490 to 7 for both TA1 and PfPK2 (Table 2; Supplementary Table 1). However, allelic diversity, summarized as mean expected heterozygosity (He) across the 10 microsatellite loci, was not significantly different among parasites in the Indian Subcontinent (mean $He = 0.78$) compared to that in Africa (mean $He = 0.76$).

Multi-locus haplotypes were constructed using predominant alleles at all of the examined loci. All 90 isolates differ from each other in at least one of the examined loci, with exception of two isolates from Africa, sharing an identical haplotype, both from Sudan. Thus, almost every isolate in each of the examined sites carried a unique genotype.

Multiplicity of infection (MOI)

Seventy-six (84.4%) out of the 90 imported P. falciparum isolates with complete set of data harbored multiple genotypes. A similar mean of multiple genotype infections was seen among parasites in the
Indian Subcontinent (84.6%) and Africa (84.4%). The minimum number of genotypes per infected person (the mean maximum number of alleles observed at all loci) was slightly lower in Africa (2.16 genotypes) than in the Indian Subcontinent (2.38 genotypes), but this was not statistically significant (P > 0.05).

**Genetic differentiation**

Alleles of most microsatellites were distributed widely across *P. falciparum* among imported malaria cases from Africa (n = 77) and the Indian Subcontinent (n = 13). However, a large number of private alleles (detected only in one region) were seen in Africa (n = 50) compared to the Indian Subcontinent (n = 5), which may reflect the smaller sample size. Nonetheless, no evidence of genetic differentiation was observed between imported *P. falciparum* from Africa and the Indian Subcontinent, ($F_{ST}=0.055$). The genetic relatedness between the *P. falciparum* populations was further illustrated by PCoA analysis (Figure 2). Analysis of molecular variance (AMOVA) revealed that the majority of the differences were due to variation between individuals within the same group (95%), and only 5% could be attributed to differences between populations.

**Distribution of drug resistance genes among imported cases**

Seventy imported *P. falciparum* isolates were examined using amplicon sequencing for four putative drug resistance genes, *Pfk13, Pfmdr1, Pfcrt* and *Pfmrp1* (Table 3). With exception of K13, there was no differences in the prevalence of wild type of the examined genes among parasites originated from Africa or Asia. There was a significantly higher prevalence of mutant *Pfk13* haplotypes among parasites from Africa than Asia. One nonsynonymous mutation in *Pfk13* (K189T) was observed at a high prevalence (36%) among parasites originating from Sudan, similar to another African countries [28]. Moreover, ten additional nonsynonymous SNPs K108E, L119L, H136N, T149S, K189T, K189N, N217H, R255K, I354V, E433D, G453A, all existed at very low prevalence ranging from 1-3% (Table 3; Supplementary Table 2).

However, the *Pfk13* mutations bringing about the amino acid substitutions C580Y, Y493H, and R539T, associated with slow artemisinin clearance of *P. falciparum* [29], were not detected among the *P. falciparum* isolates imported into Qatar. However,
**Pfmdr1** alleles encoding the polymorphisms N86Y (33%) and Y184F (77%) were prevalent among imported *P. falciparum* isolates. In addition, six rare nonsynonymous SNPs were detected (Table 3). The N86F Y184D1246 and Y86F184D1246 haplotype associated with Artemether-Lumefantrine (AL) and Chloroquine/Amodiaquine (CQ/AQ) treatment failure existed were highly prevalent among imported *P. falciparum* cases, at 43% and 33%, respectively.

Strikingly, the **Pfcrt** K76T substitution associated with CQR was found at low frequency [4/70 (6%)], however, other SNPs were seen at high prevalence, e.g. A220S (53%), Q271E (49%), N326D/S (36%), I356L (6%) and R371I (47%). The CQ sensitive haplotype C72V73M74N75K76 was common (94%), while the CQ resistant haplotypes, S72V73M74N75T76 and C72V73M74N75T76 were detected in one and three isolates, respectively.

Regarding **Pfmrp1** eight mutations were seen among imported *P. falciparum* in Qatar, ranging from high I876V (46%) to low D1533V (3%) (Table 3). The most prevalent 6 mutations (0.2 to 0.46) detected among imported cases in Qatar, have been previously reported in southeast Asian and Africa [30, 31]. **Pfmrp1** polymorphisms that have previously been associated with decreased *in vitro* susceptibility to SP, artemisinin, mefloquine, and lumefantrine were common among imported malaria in Qatar. For example, the most prevalent SNP I876V (46%), was found to be under significant selection pressure after AL treatment [30].

**Discussion**

Sustained malaria control program in GCC countries, based on case management and vector control, has resulted in elimination of malaria in the whole region, with exception of limited foci in Saudi Arabia and sporadic outbreaks in Oman [3]. However, the high influx of imported malaria, carried by young skilled and semi-skilled workers from malaria-endemic countries, maintains a risk for re-introduction of the disease [8]. The majority of imported malaria into Qatar was *P. vivax* (70.9%), while *P. falciparum* was less prevalent (26.3%). The main origin of the *P. vivax* cases was the Indian Subcontinent (83%), with a smaller proportion from Africa (17%), whereas the majority *P. falciparum* cases originated from Africa (82%), and few from the Indian Subcontinent (14%), reflecting the main malaria parasite species distribution within these regions [32]. The above findings are in line with
reported pattern of imported malaria in GCC countries [7, 33, 34], and Europe [35].

The dissemination of malaria to transmission-free areas is driven by travelers from endemic areas [35], where a large proportion of the semi-immune inhabitants sustain asymptomatic low levels parasitaemia [36, 37]. However, asymptomatic infection can develop to clinical malaria, often after a long period of settlement in a malaria-free country, that can be delayed by as long as 5 years [38] or even 8 years [39] after patients have left malaria-endemic areas. Unlike P. falciparum, P. vivax persistent liver stages (hypnozoites) may stay as dormant infection, and lead to a relapse within a period of up to 9 years after initial infection [40]. P. vivax infected travelers from endemic areas can carry parasites as undetectable hypnozoites in the liver, which can reactivate to generate a clinical relapse case months to years after individuals have left endemic areas [40]. Therefore, asymptomatic migrants with malaria parasites can maintain a long-lasting reservoir for secondary local transmission in receptive malaria-free areas, where elimination has been accomplished [3, 41].

The above is evident by the high prevalence of gametocyte carriage among imported P. falciparum malaria in Qatar. Fifty-four (74%) out of 73 imported P. falciparum isolates, successfully examined by qRT-PCR, carried gametocyte stages, with a large proportion (37%) harbouring both early and mature gametocytes, indicative of ongoing gametocytogenesis from the asexual population present in the patient, comprising a potential transmission reservoir. qRT-PCR assay of Pfpeg4 and Pfs25 are credible tools for detection of early and late gametocyte stages, respectively [42]. The density of late gametocytes among imported cases, was low compared to that reported in endemic sites, for instance among infected children in Kenya [43]. Nonetheless, low-density gametocytes can readily infect Anopheles, even at sub-microscopical levels [44, 45]. Secondary transmission, originating from imported malaria, is often reported in some GCC countries in receptive areas where the Anopheles vector is present, and a favourable ecological habitat prevails [3, 46]. The surge in mosquito abundance, in highly seasonal transmission settings, has been linked to upsurge in gametocyte numbers in asymptomatic carriers [21], in line with the hypothesis of enhanced malaria parasite infectivity in response to increased exposure to uninfected mosquitoes at the start of the transmission season [21, 47]. Although the chance of resumption of endemic transmission in GCC countries is
limited, as a result of effect vector control, the high rate of imported malaria can readily seed outbreaks in receptive areas, if vector control eases [3].

The high level of diversity among imported *P. falciparum* to Qatar; from Africa (*He* = 0.76) and the Indian subcontinent (*He* = 0.78) and genotype multiplicity parallel that reported in local parasites in both sites [48, 49], as well as sites where local transmission occurs in the region, in southwest of Saudi Arabia and Yemen [50]. The introduction of novel malaria parasites lineages into the region, via migrants from endemic areas, can enhance the parasite diversity and effective population size (*Ne*), as there is a direct relationship between the expected level of diversity and *Ne* [51]. In addition, the combination of high genotype multiplicity and gametocyte carriage, as seen in the present study, increases the likelihood that imported malaria infections will generate novel genotypes, should transmission occur. Assuming that all genotypes are readily transmissible to mosquito, good agreement has been seen between the rate of cross-mating in mosquito and the extent of multiple genotype infections in humans [52]. Thus, the imported malaria represents not only a risk for initiating local transmission, but also the ability to disseminate novel strains that can escape the effect of current drug regimen.

Consistent with the above, high prevalence of SNPs in 6 unlinked genes implicated in drug resistance were seen among imported malaria in Qatar. The success of the current efforts to prevent reintroduction of malaria in transmission-free areas, in GCC, relies on effective case management using artemisinin-combination therapy. There is significantly, high prevalence of wild type alleles among parasites in the Indian subcontinent compared to Africa. Nonetheless, known *PfK13* mutations C580Y, Y493H, and R539T, associated with slow artemisinin clearance [28] were not detected in imported *P. falciparum* for both regions. Nonetheless, many low frequency SNPs were seen, while one (K189T) was observed at a high prevalence (36%) among parasites originating from Sudan, which has been reported in other African countries [28]. Nonetheless, parasites carrying this mutation were found to have a similar response to that of wild type parasites [28] and may not impact current ACT regimen in Qatar artesunate/doxycycline or quinine/clindamycin for uncomplicated and complicated/severe *P. falciparum* malaria, respectively [11].
Therefore, the presence of SNPs linked to tolerance/resistance to artemisinin derivatives and common partners can impede this strategy, and results in persistence and increased parasite reservoir and vulnerability. For example, the high prevalence of the wild-type *Pfmdr1* alleles N86 (61%) and D1246 (97%) linked to artemether-lumefantrine (AL) resistance and the N86F184D1246 haplotype (43%) associated with AL treatment failure [53] among imported cases, could compromise the efficacy of the first line regimen (AL) for malaria in Qatar. Similarly, the commonly found *Pfmrp1* variants F1390 (79%) and I876V (46%), have been linked to decreased susceptibility to artemisinin mefloquine, and lumefantrine [30, 54]. Together, these findings demonstrate the possibility of the emergence of ACT-resistant parasites that can persist to be transmitted, even following treatment of patients with imported malaria in the region. Previous surveys in Saudi Arabia and Yemen revealed a high prevalence of drug resistance genotypes among locally acquired *P. falciparum* infection and linked the source of some of them to Africa and Indian Subcontinent [55-57].

**Conclusions**

The present study highlights the threat of imported malaria for the re-introduction of the disease in receptive malaria-free areas, such as GCC countries. The high genetic diversity and capacity of the imported *P. falciparum* to produce gametocytes, emphasis the threat of dissemination of drug resistance genotypes, that can escape current control strategies, should local transmission start. Thus, there is an urgent need for molecular tools for surveillance of imported cases, to limit the risk of re-introduction of malaria in the region.

**Abbreviations**

GCC: Gulf Cooperation Council  
HMC: Hamad Medical Corporation  
MOI: Multiplicity of infection  
qRT-PCR: Quantitative RT-PCR  
MNC: Minimum number of clones per infection  
He: expected heterozygosity He  
FST: Fixation index
Declarations

Ethics approval and consent to participate: Ethical clearance for the study was obtained from the Institutional Review Board of HMC and Weill Cornell Medicine-Qatar (Protocol no. 14-00097). A written consent was obtained from each participant before any interview or clinical examination was conducted.

Consent for publication: Not applicable.

Availability of data and materials: The datasets used in the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

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Authors’ contributions: HAB, AAS, LRC and AP conceived of the study and designed the experiments. AR, ZH, SA, AG and RN contributed to data collection and data analysis.

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Tables

Table 1: Origin of imported malaria cases in Qatar between 2013-2016. The percentage values in brackets represent the proportion of one species originating from the country listed. The information on the originating country of the expatriates was obtained in response to the questionnaire, and may not reflect all countries through which the individual travelled prior to arrival in Qatar.
| Country                  | Origin          | \(P. \text{ vivax} (%)\) | \(P. \text{ falciparum} (%)\) | \(P. \text{ vivax} + P. \text{ falciparum}\) |
|--------------------------|-----------------|---------------------------|-------------------------------|---------------------------------------------|
| The Indian Subcontinent  | India           | 148 (46.5%)               | 18 (15.3%)                    | 2                                           |
|                          | Pakistan        | 104 (32.7%)               | 4 (3.4%)                      | 0                                           |
|                          | Sri Lanka       | 0                         | 1 (0.8%)                      | 0                                           |
|                          | Nepal           | 12 (3.8%)                 | 1 (0.8%)                      | 0                                           |
| Africa                   | Mauritania      | 1 (0.3%)                  | 0                             | 0                                           |
|                          | Sudan           | 34 (10.7%)                | 36 (30.5%)                    | 4                                           |
|                          | Kenya           | 3 (0.9%)                  | 16 (13.6%)                    | 3                                           |
|                          | Nigeria         | 3 (0.9%)                  | 11 (9.3%)                     | 2                                           |
|                          | Eritrea         | 5 (1.6%)                  | 10 (8.5%)                     | 1                                           |
|                          | Ethiopia        | 5 (1.6%)                  | 3 (2.5%)                      | 0                                           |
|                          | Ghana           | 1 (0.3%)                  | 3 (2.5%)                      | 0                                           |
|                          | Rwanda           | 0                         | 2 (1.7%)                      | 0                                           |
|                          | Cameroon        | 0                         | 2 (1.7%)                      | 0                                           |
|                          | Tanzania        | 1 (0.3%)                  | 1 (0.8%)                      | 0                                           |
|                          | Djibouti        | 0                         | 1 (0.8%)                      | 0                                           |
|                          | Democratic Republic of Congo | 0 | 1 (0.8%) | 0 |
|                          | Republic of Ivory Coast | 0 | 1 (0.8%) | 0 |
|                          | Chad            | 0                         | 1 (0.8%)                      | 0                                           |
| Others*                  | Romania         | 0                         | 1 (0.8%)                      | 0                                           |
|                          | USA             | 0                         | 1 (0.8%)                      | 0                                           |
|                          | Syria           | 0                         | 1 (0.8%)                      | 0                                           |
|                          | Qatar           | 0                         | 1 (0.8%)                      | 0                                           |
|                          | Saudi Arabia    | 0                         | 1 (0.8%)                      | 0                                           |
|                          | Spain           | 0                         | 1 (0.8%)                      | 0                                           |
|                          | Canada          | 1 (0.3%)                  | 0                             | 0                                           |
| Total                    |                 | 318                       | 118                           | 12                                          |

Others*: Reported by patients who have been on visit to malaria endemic countries.

**Table 2**: Number of alleles and expected heterozygosity \((He)\) at ten microsatellite loci among imported *Plasmodium falciparum* from the Indian Subcontinent and Africa.
Table 3. Haplotypes of drug resistance genes, that exist at a prevalence of more than 5%, among imported *P. falciparum* to Qatar. Haplotypes are shown as amino acids (wild-type in normal case, substitutions in **bold underlined**).

| Locus | Genotype | Haplotype | Prevalence | Asia (n=7) | A |
|-------|----------|-----------|------------|------------|---|
| *Pfcrt* | Wild type | C72 K76 A220 Q271 N326 I356 R371 | 0.44 | 1(0.14) | 3 |
|        | Mutant | C72 K76 S220 E271 N326 I356 R371 | 0.13 | 6(0.85) | 3 |
|        | Mutant | C/S72 T76 S220 E271 N326 I356 R371 | 0.05 |  |  |
|        | Mutant | C72 K76 S220 E271 S326 I356 R371 | 0.29 |  |  |
| *Pfmdr1* | Wild type | N86 F184 F938 G968 D1246 | 0.39 | 4(0.57) | 2 |
|        | Mutant | N86 Y184 F938 G968 D1246 | 0.1 | 3(0.43) | 4 |
|        | Mutant | Y86 F184 F938 G968 D1246 | 0.3 |  |  |
| *Pfk13* | Wild type | H136 T149 K189 N217 R255 E433 G453 | 0.47 | 7(1.00) | 2 |
|        | Mutant | H136 T149 T189 N217 R255 E433 G453 | 0.34 | 0(0.00) | 3 |
| *Pfmrp1* | Wild type | H191 K202 S437 L876 L1342 F1390 K1466 D1533 | 0.31 | 1(0.14) | 2 |
|        | Mutant | H191 K202 S437 V876 L1342 F1390 K1466 D1533 | 0.06 | 6(0.86) | 4 |
|        | Mutant | H191 K202 S437 V876 L1342 F1390 K1466 D1533 | 0.1 |  |  |
|        | Mutant | Y191 K202 A437 V876 L1342 F1390 K1466 D1533 | 0.05 |  |  |
Correlation between total parasite density with both early gametocyte and late gametocyte density. (A) log total parasitemia (X axis) and log early gametocyte density (Y axis), the fit line in scatter plot shows a weak/non-significant correlation coefficient ($r = 0.031$, $p = 0.835$). (B) log total parasitemia (X axis) and log late gametocyte density (Y axis), the fit line in scatter plot shows a weak/non-significant correlation coefficient ($r = 0.008$, $p = 0.946$).
Figure 2

Principal Co-ordinates Analysis (PCoA) of P. falciparum populations in two regions (the Indian Subcontinent and Africa). Values within () after the coordinate number, are the percentage of variation explained by the coordinate.

Supplementary Files

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Supplementary Table 1.docx
Supplementary Table 2.docx