Genetic diversity of *Plasmodium vivax* among immigrant patients exhibiting severe and non-severe clinical manifestations in northern suburbs of Paris

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ABSTRACT

*Plasmodium vivax* is the most frequent and widely distributed cause of recurring malaria. It is a public health issue which mostly occurs in South-East Asia, followed by Middle East, Latin and South...
Americas and sub-Saharan Africa. Although it is commonly known as an etiologic agent of malaria with mild clinical manifestations, it can lead to severe complications. It has been neglected and understudied for a long time, due to its lower mortality, culturing infeasibility and mild clinical manifestations in comparison to *P. falciparum*. Despite the mild clinical issues commonly raised for *P. vivax*, the correlation between the clinical manifestations exhibited by the patients with severe and non-severe complications and the genetic diversity of the parasites responsible of the disease is not clear. An investigation was carried out between 2011 and 2021 on the patients referred to Avicenne hospital for suspected *P. vivax* infection. At arrival, they were undergone for clinical and biological examinations. The lateral flow test and LAMP-PCR confirmed the presence of malaria parasites, *Plasmodium* sp.. Microscopic examination revealed the presence of *Plasmodium* parasites with a parasitaemia between 0.01 to 0.38%. Conventional PCR amplifications targeting 714 bp DNA fragment of small subunit ribosomal DNA (SSU-rDNA) followed by bidirectional sequencing allowed us to identify the parasites as *P. vivax*. The neighbor-joining phylogenetic tree revealed that *P. vivax* sequences processed in the present study clustered in two well-differentiated and supported clades. It included a bigger clade including *P. vivax* specimens of all our patients together with homonymous sequences from Indonesia, India and El Salvador and the second clade encompassed the sequences from Yemen and India. In addition, the clustering displayed by the median-joining network agreed well with the topology of the phylogenetic tree generated by the neighbor-joining analysis. No correlation between the clinical manifestation of the patients with severe and non-severe complications, encompassing diverse geographical origins, and genetic diversity of the parasites was observed, since all sequences demonstrated a high homogeneity. These findings can be helpful in getting the knowledge about the population genetics of *P. vivax* and taking the proper control management strategies against these parasites.

**Keywords:** *Plasmodium vivax*, phylogenetic analysis, genetic diversity, severe malaria complication
INTRODUCTION

Malaria is a life-threatening vector-borne disease caused by protozoa of the genus *Plasmodium*, transmitted by the infected females of *Anopheles* species [1]. Up to the present, 5 species including *Plasmodium falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi* are known as responsible of disease in humans [2]. Malaria is a public health issue in which nearly half of the world's population living in 87 countries and territories are at risk [3,4]. According to WHO 2020 report, the number of malaria cases is estimated about 229 millions in 2019 with a mortality amounted to 409,000 deaths [3,4]. It mostly occurs in sub-Saharan Africa followed by South-East Asia, Eastern Mediterranean, Western Pacific, and Americas [5,6].

*Plasmodium vivax* is the most frequent and widely distributed cause of recurring malaria. It is mainly prevalent in central, South and Southeast Asia, Middle East, Latin and South America and in some restricted parts of Africa with almost 2.85 billion people at risk [7,8]. It is commonly responsible for the mild symptoms, including fever, headache, chills or sweating [9]. These mild clinical manifestations are attributed to the fact that *P. vivax* infects only the young erythrocytes against *P. falciparum* which infects all stages of erythrocytes [10]. Nevertheless, it can cause a severe form of malaria with atypical symptoms including respiratory distress, ruptured spleen, renal failure, retinal hemorrhage, severe anemia, thrombocytopenia, hemoglobinuria or cerebral complications [11,12]. *Plasmodium vivax* malaria has been overlooked over the time, because of its mild characteristics and lower mortality rate compared to severe *P. falciparum* malaria [13].

In order to implement effective strategies against malaria, accumulating knowledge on the genetic structure of the parasites isolated from the infected individuals is essential which helps to better understand the local patterns of malaria transmission and the dynamics of genetic recombination in natural *P. vivax* populations. This genetic diversity in *P. vivax* parasites can be affected by some factors such as demography of the infected populations, migration, genetic recombination or evolutionary history of the parasite [14].

Despite several investigations carried out on the genetic diversity of *P. vivax* worldwide, most of them provided fragmentary information in the restricted areas and only four studied the genetic diversity and population structure of this parasite on a worldwide scale [15,16,17,18]. In addition, none of them argue about the probable correlation between this genetic diversity within *P. vivax* and the clinical manifestations exhibited by the patients. The aim of present study was to determine the probable correlation between the clinical manifestations appeared in a case series of the patients infected by *P. vivax*, referred to Avicenne hospital (Bobigny, France), and the inter- and intraspecific variations and the genetic diversity within *P. vivax* isolates coming from diverse geographical areas. The latter helps to understand if genetic diversity of the parasites has an impact on the severity of
clinical manifestations exhibited by the patients.

MATERIALS AND METHODS

Samples and clinics

The investigation was conducted between 2011 and 2021 on the suspected patients referred to Avicenne hospital (northern suburb of Paris) for probable *P. vivax* infection. At arrival, they were undergone for clinical and biological examinations. The venous blood (5 mL) was collected in EDTA vacutainers from individuals with clinical symptoms reminiscent of severe and non-severe *P. vivax* malaria for diagnosis thorough parasitological and molecular analyses. The demographic (e.g., gender, age, location, and occupation) and clinical (medical antecedent, prescribed medication, travel history to endemic regions, and probable prophylactic measures) information were recorded for each patient individually.

Parasitological diagnosis

May-Grünwald-Giemsa stained thin and thick blood smears were prepared from peripheral blood of the patients and stained with 10% Giemsa for 20 minutes. They were examined under a light microscope (1000× magnification) to identify malaria parasites. Parasitemia was defined as the number of parasites detected per 10,000 red blood cells (RBCs) in a thin blood smear [19]. The microscopic examination of the isolates was further accompanied by LAMP-PCR (Alethia® Malaria, Meridian Bioscience) and lateral flow test (BinaxNOW® Malaria, Abbott, USA; VIKIA® Malaria Ag Pf/Pan, Biomérieux, France) to diagnose *Plasmodium* sp. infection [20,21].

Molecular characterization and typing

In order to investigate molecular characterization, genetic diversity and population structure of the parasites isolated from the patients in correlation with their exhibited clinical symptoms, the malarial parasitic DNA was extracted from peripheral blood samples using a Qiagen DNA blood kit (Qiagen, Hilden, Germany) according to the manufacturer’s protocol. It was then subjected to conventional PCR targeting small subunit ribosomal DNA (SSU-rDNA) gene, using forward (rPV1: 5'-CCGAATTCAGTCCCACGT-3') and reverse (rPV2: 5'-GCTTCGGCTTGGAAGTCC-3') primers with an expected length of 714 bp. Each reaction included 25 µL master mixture, containing 12.5 µL Mastermix (AmpliTaq Gold 360, Applied Biosystem), 8 µL DDW, 1 µL of each primer and 2.5 µL of template DNA. A total of 35 cycles was performed by a PCR-Thermal-Cycler (Applied Biosystem, USA), under the following conditions: initial denaturation for 5 min at 95°C, followed by 35 cycles of 94°C for 1 min, 55°C for 2 min, 72°C...
for 90s, and final extension at 72°C for 5 min [22]. Double-distilled water and already purified DNA isolated from *Plasmodium vivax* patient were used as negative and positive controls for each PCR batch. Amplicons were analyzed using electrophoresis in a 1.5% agarose gel containing ethidium bromide. PCR products were purified using an Invisorb Fragment CleanUp kit (Stratec Molecular, Berlin, Germany) and sequenced using the same primers for PCR amplification. The obtained sequences were edited, aligned, and blasted with GenBank database sequences to identify *Plasmodium* species. The sequences were compared to homologous sequences collected in the GenBank database and aligned with the Basic Local Alignment Search Tool (BLAST) (www.ncbi.nlm.nih.gov/BLAST). All sequences were identified based on ≥99% identity with GenBank sequences. Sequence alignment of amplified fragments using BioEdit allowed us to look for the nucleotide polymorphisms. The phylogenetic analysis was carried out using MEGA v.6 software [23]. A SSU-rDNA phylogenetic tree of *Plasmodium* isolates (identified in this study) and GenBank sequences was constructed using neighbor-joining (NJ) and the p-distance substitution model, supported by bootstrap values of 1000 replicates. To display the genetic relationships within *Plasmodium* populations, the median joining algorithm was implemented using NETWORK v. 5 software [24].

**RESULTS**

A total of 13 isolates were analyzed by clinical, parasitological and molecular examinations. They belonged to 12 patients, one patient (AVC4) with a relapse. They were originally from Afghanistan (4 cases), Pakistan (4), France (2), Sudan (1) and India (1). The patients had an average age of 32 years old, mostly between 20 to 45 years old. The men were the most predominant patients (10 men against two women). Detailed epidemiological and clinical information of the patients are given in the Table 1. Based on the clinical examinations, three patients (AVC2, AVC4 and AVC6) exhibited the clinical symptoms resembling severe malaria while nine patients possessed non-severe malaria according to the WHO recommendation [3,25]. Diffuse intravascular coagulation (DIC), Macrophagic Activation Syndrome (SAM) and septic shock were such of the symptoms observed in the patients with severe malaria. All of the patients had a parasitaemia inferior to 0.4%. No correlation was observed between the level of parasitaemia and the severity of clinical pictures, but the severe cases were among the patients with highest parasitemia (0.3 to 0.38%). Clinical symptoms observed in the patients with severe versus non-severe *P. vivax* malaria is given in Table SI-1.

Microscopic examinations revealed the infection of the patients with *P. vivax*. The morphological identification was carried out based on some criteria such as enlarged infected erythrocytes and the appearance of granules, called 'Schüffner's dots', over the erythrocyte cytoplasm. Parasitological
analyses by LAMP-PCR and lateral flow test were further confirmed the infection by *Plasmodium* sp. parasites.

In order to confirm the identity of the parasites and to determine inter- and intraspecific genotypic relationships between our isolates and those reported from other endemic regions, the *Plasmodium* isolates were subjected to conventional PCR targeting SSU-rDNA. All isolates found positive after microscopic examination, were also positive by PCR. Bidirectional sequencing allowed identifying the parasites at the species level as *P. vivax*. All the sequences were deposited in GenBank under the assigned accession numbers of XK542981 to XK542994.

Based on NJ phylogenetic tree generated from our sequences and those from Genbank, the *P. vivax* isolates were clustered in two well-differentiated and supported clades (Figure 1). The first bigger clade included *P. vivax* specimens of all our patients together with homonymous sequences from Indonesia (GU233451), India (JQ627153- JQ627155, JQ627158, and GQ477744) and El Salvador (XR-003001206, XR-003001217, XR-003001225 and U07367). The second clade encompasses the sequences from Yemen (HQ283224, and HQ283225) and India (JQ627157, JQ627156 and JQ627157). The sequence alignment of the isolates revealed the presence of two SNPs (single nucleotide polymorphism) which explains the presence of two subpopulations of *P. vivax* in some countries such as India (Figure 2). In addition, the clustering displayed by the median-joining network was in accordance with the topology of the phylogenetic tree generated by the neighbor-joining analysis (Figure 3). The distribution of SSU-rDNA haplotypes within *P. vivax* sequences processed in the present study is shown in Table 2.

**DISCUSSIONS**

France has one of the highest numbers of malaria cases reported in returned travellers, with about 5000 cases per year [26,27]. Around 95% of the malaria cases are observed in people returning from malaria-endemic countries [27]. Patients with *P. vivax* make up 4% of the total number of imported cases [12]. In the present study, 10 out of 12 patients were the immigrants from endemic countries mostly from Afghanistan and Pakistan. Two cases were French traveler patients with a history of recent travel to Pakistan, Iraq and Yemen. Furthermore, most of the processed individuals were men which points to the fact that the majority of immigrants are men [28](Table 1).

*P. vivax* malaria is known to possess the mild complications [10]. With less than one severe case per year in average, *P. vivax* is very rarely associated with severe imported malaria in France [12]. Dramatically, three out of 12 patients were exhibited clinical symptoms such as Macrophagic Activation Syndrome (SAM), diffuse intravascular coagulation (DIC) and septic shock which based on RPC 2017 and WHO 2020 [3,25] recommendations, two latters implying to severe malaria.
Although some other complications like impaired consciousness, respiratory distress, multiple convulsions, prostration, pulmonary oedema, abnormal bleeding or jaundice have been reported for *P. vivax* severe malaria [29], they were absent in our patients. Therefore, in contrary to the benign reputation of *P. vivax* malaria, its clinical manifestation is not always very mild, inciting acute infection with septic shock, SAM or DIC. Furthermore, one of the patients with Pakistani origin exhibited a relapse one year after the first infection (Table 1). Except mentioned patient, no case of multiple infections from same individual was noticed in this study. In endemic areas, relapse of *P. vivax* malaria is a major cause of malaria in young children, and an important source of malaria transmission which can be appeared even more than 5 years after initial contamination [30,31]. Furthermore, most of the patients had a history of local travel to countries of their homeland.

The malaria epidemiology is influenced by environmental factors (e.g., temperature, rainfall) and socioeconomic conditions. Besides, other factors such as urbanization, exponential population growth, instability, military conflicts, migration, and environmental changes due to excessive rains or floods, and extensive irrigation projects favor malarial parasite transmission as well [32]. Afghanistan and Pakistan are the endemic foci with high burden of malaria. The eco-geographical diversity in Afghanistan contributes to the heterogeneous prevalence of malaria across the country. Approximately 60% of the population (nearly 14 million people) lives in malaria-endemic areas [33]. Eighty-five percent of the whole malaria cases are prevalent at 63 out of 400 districts. Most of them (Nangarhar, Kunar, Nuristan, Khost, Paktika and Laghman) are located along the border with Pakistan [34]. *Plasmodium vivax* is the prominent species in Afghanistan causing more than 95% of all malaria cases [35]. Military conflicts and instability together with living at unsuitable locations, lack of means for personal protection, difficulty of access to health care are of such elements which favor the emergence of vector breeding sites, population movements and malaria high burden. In Pakistan, malaria is one of the most devastating parasitic diseases with 110 million individuals at risk and an estimated incidence of 500,000 cases and 50,000 deaths annually. *P. vivax* is the most prevalent species (88%) followed by *P. falciparum* (12%) [36]. According to the latest stratification, 66 districts have been categorized in the high endemicity stratum (Annual Parasite Incidence>5 per 1000) in which those located in the northern part of the country (e.g., Federally Administered Tribal Areas (FATA), Baluchistan and Khyber Pakhtunkhwa (KP) provinces) possess the highest burden of Malaria [36,37,38]. Many factors have contributed toward increase of malaria cases in these regions including warm autumns (resulting in extended transmission period), emergence of chloroquine resistance across the country and a chronic decline in vector control activities [39,40]. In addition, the migration of peoples from malarial endemic regions to less or non-immune communities can lead to the serious threat of malaria reintroduction in malaria free-areas [41,42]. Five out of eight patients from
Afghanistan and Pakistan in this study come from this buffer endemic zone on the Afghan-Pakistani border.

Despite wide distribution of *P. vivax* as the most frequent species worldwide, a comprehensive picture of the global genetic diversity and population structure of *P. vivax* has been poorly studied. Although several local investigations have been conducted on the genetic diversity of *P. vivax*; but they provide only a piece of fragmentary information without giving a global image. In this descriptive-analytic study, we aimed to evaluate the correlation between genetic diversity of *P. vivax* and the clinical symptoms of the patients with severe and non-severe infections. We therefore did not evaluate the factors associated with pathogenicity or severity of causative *P. vivax* by molecular analysis. Despite relatively low specimen numbers examined, we found a genetic diversity among our isolates comparing to those from other endemic countries. In analyzing the NJ phylogenetic tree generated with specimens of our patients together with GenBank sequences, we recorded a genetic heterogeneity among the processed sequences leading to cluster with their counterparts in two clades (Figure 2). These finding are consistent with the results of other studies indicating the presence of two lineages categorized as Old World and New World, based on geographical sub-division and genetic and phenotypical markers. These lineages are not confined geographically and are present worldwide [43]. In other investigation on the *P. vivax* patients in Southern Thailand, high level of genetic diversity within *P. vivax* specimens was also reported using three antigenic markers and eight microsatellite markers [44]. Afghanistan and Pakistan isolates demonstrated to have a high homogeneity while a degree of genetic separation was observed for some isolates from India (Table 2). This finding supports the results of Benavente et al. [18] in which Afghanistan and Pakistan isolates were clustered together in the same clade. Unlike, despite the geographically close distance, the highest genetic diversity in *P. vivax* isolates was observed in the sequences from India which led to the grouping of Indian samples in two sub-populations. A genetic separation was observed in some Indian isolates comparing to Pakistan and Afghanistan’s specimens [18]. In the study conducted by Rougeron et al. [16], Pakistan’s isolates were positioned in a clade far from Indian isolates. Nevertheless, this genetic separation was valid for some of our samples, not for all. Consequently, the correlation between genetic diversity and geographic distance from central Asia (India) remained highly significant [17]. On the other hand, no correlation between the clinical manifestation of the patients with severe and non-severe complications and genetic diversity of the parasites was observed, since all sequences demonstrated a high homogeneity (Figure 2). In sequence alignment of the isolates processed in this study with those coming from Genbank, two SNPs were observed which explains the presence of two subpopulations of *P. vivax* in some countries such as India (Figure 2). These findings were further supported by Network analysis (Figure 3).
The patients processed in the present study were undergone the treatment with Chloroquine (10 mg/kg oral tablet in d1 and d2, and 5 mg/kg in d3) for non-severe *P. vivax* malaria and Quinine (25mg/kg/d), and Artesunate (2.4mg/kg), for severe cases and a favorable outcome was observed within 7 days post-treatment (Table 1).

CONCLUSION

Although *P. vivax* is known as a pathogen with mild clinical manifestations, we provide further evidence on the exhibition of severe complications (Diffuse intravascular coagulation (DIC), Macrophagic Activation Syndrome (SAM) and septic shock) in 3 out of 12 studied patients. In addition, we highlight a heterogeneity within isolated parasites using NJ phylogenetic analysis in which the *P. vivax* sequences clustered in two well-differentiated and supported clades. The first clade includes *P. vivax* specimens of all our patients together homonymous sequences from India, Indonesia and El Salvador and the second clade encompasses the sequences from Yemen and India. Furthermore, no correlation between the clinical manifestations of the patients with severe and non-severe complications and genetic diversity of the parasites was observed. However, these findings are limited to restricted number of the patients analyzed in this study. These results can be supported by a larger scale sampling in terms of geographical locations from France and possibly other endemic countries, by evaluating other demographic factors (e.g., sex, age) and by looking for other molecular markers particularly in relation with pathogenicity of these parasites.

DECLARATIONS

Ethical statement

All clinical procedures, including the protocols for the collection of patients’ blood samples were approved by Avicenne Hospital Ethics and Research Committee, protocol number of 95/99/FR-EC128/ESA.

Consent for publication

All adult subjects provided written informed consent, and the father of child participant provided an informed consent on their behalf.

Availability of data and material

Not applicable.

Competing interests
The authors declare no conflicts of interest.

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Authors' contributions

Conceptualization: AI, and MA; Clinical and molecular diagnosis: AS, OH, AM, SB, OB, YC, FA, MT, SH, AI and MA; writing—original draft preparation: MA; writing—review and editing: AI, SH, and MA. All authors have read and agreed to the published version of the manuscript.

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Table 1. Epidemiological and clinical details of *Plasmodium vivax* infected patients analyzed in the present study

<table>
<thead>
<tr>
<th>Patient name</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Origin</th>
<th>Travel history</th>
<th>Clinical symptom</th>
<th>Parasitemia</th>
<th>Treatment*</th>
<th>Clinical Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>AVC1</td>
<td>M</td>
<td>33</td>
<td>Pakistan</td>
<td>Local travel</td>
<td>Fever</td>
<td>0.01</td>
<td>Chloroquine (10 mg/kg oral tablet in d1 and d2, and 5 mg/kg in d3)/Atovaquone-Proguanil Hcl (4*250mg-100mg/day for 3 days)</td>
<td></td>
</tr>
<tr>
<td>AVC2**</td>
<td>M</td>
<td>27</td>
<td>Afghanistan</td>
<td>Local travel</td>
<td>Abdominal pain, Headache, Fever, Septic shock, DIC</td>
<td>0.30</td>
<td>Artesunate (2.4mg/kg)/Chloroquine (10 mg/kg oral tablet in d1 and d2, and 5 mg/kg in d3)</td>
<td></td>
</tr>
<tr>
<td>AVC3</td>
<td>F</td>
<td>56</td>
<td>Pakistan</td>
<td>Local travel</td>
<td>Fever, Swear, Abdominal pain, Vomiting, Headache</td>
<td>0.15</td>
<td>Chloroquine (10 mg/kg oral tablet in d1 and d2, and 5 mg/kg in d3)</td>
<td></td>
</tr>
<tr>
<td>AVC4**</td>
<td>M</td>
<td>24</td>
<td>Pakistan</td>
<td>Germany</td>
<td>Grippal syndrom, Abdominal pain, Vomiting, SAM</td>
<td>0.22</td>
<td>Quinine (8mg/kg)/Piperaquine-Artenimol (4*320mg-40mg/day for 3 days)</td>
<td></td>
</tr>
<tr>
<td>AVC5</td>
<td>M</td>
<td>20</td>
<td>Sudan</td>
<td>Local travel</td>
<td>Abdominal pain, Fever, Headache, Dark urine</td>
<td>0.12</td>
<td>Chloroquine (10 mg/kg oral tablet in d1 and d2, and 5 mg/kg in d3)</td>
<td></td>
</tr>
<tr>
<td>AVC6**</td>
<td>M</td>
<td>25 (AVC4 relapse)</td>
<td>Pakistan</td>
<td>Local travel</td>
<td>Abdominal pain, Headache, Fever, Septic shock</td>
<td>0.38</td>
<td>Quinine (8mg/kg)/Piperaquine-Artenimol (4*320mg-40mg/day for 3 days)</td>
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<tr>
<td>AVC7</td>
<td>F</td>
<td>45</td>
<td>France</td>
<td>Pakistan</td>
<td>Headache, Swear</td>
<td>0.05</td>
<td>Chloroquine (10 mg/kg oral tablet in d1 and d2, and 5 mg/kg in d3)</td>
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<tr>
<td>AVC8</td>
<td>M</td>
<td>34</td>
<td>Afghanistan, Iran, Turkey, Greece, Italy</td>
<td>Local travel</td>
<td>Fever, Headache, Abdominal pain, Vomiting</td>
<td>0.16</td>
<td>Piperaquine-Artenimol (4*320mg-40mg/day for 3 days)</td>
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<td>AVC9</td>
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<td>24</td>
<td>Afghanistan</td>
<td>Local travel</td>
<td>Fever, Headache, Asthenia, Confusion (Chloroquine intolerance)</td>
<td>0.30</td>
<td>Quinine (8mg/kg)/Piperaquine-Artenimol (4*320mg-40mg/day for 3 days)</td>
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<td>M</td>
<td>23</td>
<td>India</td>
<td>Local travel</td>
<td>Fever, Swear, Headache</td>
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<tr>
<td>AVC11</td>
<td>M</td>
<td>27</td>
<td>Pakistan, Iran, Turkey, Balkan</td>
<td>Local travel</td>
<td>Fever, diffused pain, conjunctival icterus, Headache</td>
<td>0.06</td>
<td>Chloroquine (10 mg/kg oral tablet in d1 and d2, and 5 mg/kg in d3)</td>
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<tr>
<td>AVC12</td>
<td>M</td>
<td>67</td>
<td>France</td>
<td>Iraq, Yemen</td>
<td>Asthenia, Headache, Fever, diffused pain</td>
<td>0.30</td>
<td>Piperaquine-Artenimol (4*320mg-40mg/day for 3 days)</td>
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<tr>
<td>AVC13</td>
<td>M</td>
<td>8</td>
<td>Afghanistan</td>
<td>Local travel</td>
<td>Vomiting, Fever, Swear</td>
<td>0.05</td>
<td>Artemether-Lumefantrine- (3*20mg: 120mg/12h for 2.5 days)</td>
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</table>

*: All of the processed patients were treated with primaquine (30mg/d for adults and 0.5mg/kg/d for infant for 14 days) as a complementary treatment to avoid the relapse; **: Patients with severe *P. vivax* malaria; DIC: Diffuse intravascular coagulation; SAM: Macrophagic Activation Syndrome
Table 2. Distribution of SSU-rDNA haplotypes within the *P. vivax* populations analyzed in this study

<table>
<thead>
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<th>Haplotype</th>
<th>Numbers</th>
<th>Country</th>
<th>Total</th>
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<tr>
<td>H1</td>
<td>25</td>
<td>Salvador</td>
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<td></td>
<td></td>
<td>India</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unknown</td>
<td>1</td>
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<tr>
<td></td>
<td></td>
<td>Pakistan</td>
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<td></td>
<td></td>
<td>Afghanistan</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Indonesia</td>
<td>1</td>
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<tr>
<td></td>
<td></td>
<td>France</td>
<td>1</td>
</tr>
<tr>
<td>H2</td>
<td>5</td>
<td>India</td>
<td>3</td>
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<td>Yemen</td>
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<tr>
<td>Total</td>
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