Situation of antimalarial drug efficacy and resistance: focus on special cases

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• Definitions
• Artemisinin partial resistance(s)
• Case reports
• Piperaquine resistance in Africa
• Advice on data sharing, methods to assess origin of parasites and QC of circulating DHAPIP
• **Antimalarial resistance** is defined as the ability of a parasite strain to survive and/or multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within tolerance of the subject;

• **Multidrug resistance (MDR)** is resistance to more than 2 antimalarial compounds of different chemical classes. This term usually refers to *P. falciparum* resistance to chloroquine, sulfadoxine-pyrimethamine, and a third antimalarial compound;

• **Artemisinin resistance** is defined as delayed parasite clearance following treatment with an artesunate monotherapy or with an ACT – partial resistance would be more appropriate wording;
Distribution of C580Y mutations worldwide

Possible “permissive” or compensatory background mutations

Miotto et al., Nature Genetics 2015
### Relation between partner drug efficacy and K13 mutations

<table>
<thead>
<tr>
<th>Year</th>
<th>Site</th>
<th>ACT</th>
<th>N</th>
<th>Efficacy 28/42 days (%)</th>
<th>K13 mutant (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2016</td>
<td>Kampong Speu, Kratie</td>
<td>Artesunate-mefloquine</td>
<td>69</td>
<td>100</td>
<td>95.6 (C580Y)</td>
</tr>
<tr>
<td>2017</td>
<td>Kampong Speu, Pursat, Stungtreng</td>
<td>Artesunate-mefloquine</td>
<td>170</td>
<td>99.5</td>
<td>78.2 (C580Y, R539T, Y493H)</td>
</tr>
<tr>
<td>2017</td>
<td>Ratanakiri, Mondulkiri</td>
<td>Artesunate-pyronaridine</td>
<td>123</td>
<td>97.6</td>
<td>72.4 (C580Y)</td>
</tr>
<tr>
<td>2017</td>
<td>Kachin, N. Shan</td>
<td>Artemether-lumefantrine</td>
<td>71</td>
<td>97.2</td>
<td>43.7 (F446I, R561H)</td>
</tr>
</tbody>
</table>

**Even if delayed clearance doesn’t directly lead to treatment failure**, it puts more pressure on partner drugs to succeed in mopping up lingering parasites, says Nicholas White, a professor of tropical medicine at Mahidol University and the University of Oxford.

**Are We Headed for a New Era of Malaria Drug Resistance?**
[https://www.the-scientist.com/features/are-we-headed-for-a-new-era-of-malaria-drug-resistance--65496](https://www.the-scientist.com/features/are-we-headed-for-a-new-era-of-malaria-drug-resistance--65496)
Role of each markers in DHA-PIP efficacy in Cambodia (N = 725)

K13 WT PIP WT (n=268)
K13 WT PIP MUT (n=14)
K13 MUT PIP WT (n=208)
K13 MUT PIP MUT (n=235)

Witkowski et al., Lancet Inf. Disease 2016
Artemisinin partial resistance: Guyana

**Articles:**


**Summary:**

- Samples collected in 2010 for *Pfhrp2* survey;
- 5/98 samples carried the mutant C580Y (4/5 from zone 7 and 1/5 zone 1).
- All five samples had similar *Pfkelch 13* flanking microsatellite profiles and were different to the ones observed in Southeast Asia;
Artemisinin partial resistance: Guyana

Actions taken:

• TES between June-Nov 2014:
  • 7-day artesunate trial (4 mg/kg/day) + primaquine single dose;
  • n = 50 (26% from zone 1; 54% zone 7; 16% zone 8);
  • day3+ rate = 2%; 100% efficacy and 100% Pfkelch 13 wild type.

• Survey conducted between 2016-2017 whole country (n = 877)
  • presence of C580Y mainly in zone 1;
  • declining trend over time.
Prevalence of *Pfkelch13* C580Y by region in Guyana

<table>
<thead>
<tr>
<th>Region</th>
<th>N</th>
<th>C580Y</th>
<th>% mutant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Region 1</td>
<td>114</td>
<td>10</td>
<td>8.8</td>
</tr>
<tr>
<td>Region 2</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Region 3</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Region 7</td>
<td>572</td>
<td>3</td>
<td>0.5</td>
</tr>
<tr>
<td>Region 8</td>
<td>150</td>
<td>1</td>
<td>0.7</td>
</tr>
<tr>
<td>Region 9</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Region 10</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Venezuela</td>
<td>21</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Unspecified</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>877</td>
<td>14</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Prevalence of *Pfkelch13* C580Y by region in Guyana
Trend over time of \textit{PfKelch 13 C580Y} in Guyana
Artemisinin partial resistance: Guyana

Actions taken cont’d:
• To understand origin of those mutants:
  • nine microsatellite loci flanking the *Pfkelch 13* gene, whole genome sequencing (WGS) and selective whole genome amplification (SWGA) were performed;
  • confirm that the *Pfkelch 13* C580Y variant arose on a single Guyanese haplotypic background, and was not imported from South-East Asia.

• TES in June-Oct 2018 in Georgetown and zone 1:
  • Georgetown: completed (n = 84); microscopy QC needed; *Pfkelch 13* absent in Georgetown (n = 99);
  • Zone 1: on-going.
Artemisinin partial resistance: Papua New Guinea

Summary:
- Sampling periods: 11-30 January 2016 (n = 112) and 23 January-11 February 2017 (n = 132);
- Symptomatic patients > 2 years with *P. falciparum* confirmed by RDT or microscopy;
- In 2017, 3/132 patients carried C580Y vs 0/112 in 2016;

Articles:
Artemisinin partial resistance: India

Articles:


Summary:
• TES in West Bengal (4 sites) between **2014-2016**, n = 226;
• ASSP failure rate = 15.9% (7.9% ETF) and all treatment failures cured with AL;
• Parasite clearance time > 5.0 h  = 11.9%; based on the analysis of *Pfkelch 13* and *pfmdr1* 184F + 1042D which role is unclear;
• *Pfkelch 13* F446I (n = 2), R539T (n = 7), G625R (n = 21), and N672S (n = 4) were identified in 34/226 (15%) isolates;
• Among 7 patients with R539T allele, 5 patients were working in Cambodia during the past 15 days.
Artemisinin partial resistance: India

Comments:

Several issues challenge the conclusion:

• Inadequate definitions used:
  • ETF ≠ artemisinin resistance; extremely high parasitemia at day 3;
  • confusion between candidate/validated Pfkelch 13 mutant and suspected/confirmed artemisinin resistance;
  • in vitro threshold (1% not 10%);
  • parasite clearance half-life threshold (5.5 h not 5.0 h);
• G625R is neither validated nor a candidate marker for artemisinin partial resistance; only reported once in Gabon;
• Data contrast with other available data: ASSP efficacy between 2010-2017 (n = 52) is on average 99.3% (93.8-100%) and 100% in West Bengal in 2014;
• Survey in India (2014-2016) (n = 832) PfKelch13 mutations = 1.4% (different from 15% reported);
• R539T has almost disappeared in Cambodia after 2014 (2.1% in 11 studies in 2014).

Actions taken:

• QC (slides, DNA, sequencing) and re-analysis requested but rejected by the authors;
• G625R genome editing and RSA_{0-3h} studies;
• TES studies in West Bengal with parasites clearance and Pfkelch 13 analysis.
Artemisinin partial resistance: Equatorial Guinea

Article:


Summary:

• 43-year-old man Chinese worker returning from Equatorial Guinea in 2013 and developing a malaria attack in China treated successfully with DHA-piperaquine. Day 3 parasitaemia: 40/ml (1/200 WBC); RSA0-3h survival rate ≈ 2%, PfKelch13: M579I (confirmed by IPC).

• Origin confirmation led to controversy;
  • The analysis was based on 26,918 SNPs spanning the entire genome. The SNP/REF were extracted from the 26,918 positions that differentiate 245 Plasmodium samples into their respective geographical origins;
  • CWX sample had a total of 559 SNPs predicted by samtools mpileup;
  • Geographic origin was also independently confirmed based on a 23-SNP barcode within the apicoplast and mitochondrial genomes.
Comments:

- Period of 8 weeks between return from Africa;
- only 1 case of M579I was previously reported in Myanmar;
- *PfKelch 13* mutations frequently appear and disappear due to fitness cost. A single case cannot lead to the statement that resistance has emerged in a country or continent (ref. WHO definition on artemisinin resistance);
- Two studies conducted in 2005 and 2013-2014 (n = 98 + 144) in Equatorial Guinea did not report M579I.

Actions taken:

- RSA$_{0-3h}$ after gene editing M579I $\approx$ 10%
- TES in 3 sites with 2 arms (AL & ASAQ) in 2017-2018: n = 438; day 3 positivity rate = 0%;
- *PfKelch 13* on-going (final results expected soon; 100 samples absence of M579I).
Artemisinin partial resistance: Rwanda

Article:

Summary:
• A total of 534 children were treated with AL (n=267) or DHP (n=267) in 2 sites: Masaka and Ruhaha (2013-15);
• After PCR adjustment, 98.3% ACPR in the AL at day 28 and 98.4% ACPR for DP at day 42;
• Day 3+ rate ranged from 0.8 to 2.5%.
Artemisinin partial resistance: Rwanda

Comments:

• Analysis of K13 was performed on TES samples from 2012-15 (AL in 4 sites Bugarama, Kibirizi, Nyarurema, and Rukara) and 2013-15 samples (Masaka and Ruhaha);

• Total of 927 samples among which 45 had non-synonymous mutations;

• R561H was found in 20 samples: 19 in Masaka (7.3%) and 1 in Rukara;

• No correlation between R561H and day3+ and not correlation between 561 and treatment failure (among the 9 treatment failures 8 were reinfection and 1 ETF = low parasitemia at day3 + fever) (data to be confirmed).

Actions taken:

• Whole genome sequencing on the parasite to evaluate a clonal expansion;

• Requested individual patient data from country;

• TES conducted in 2018 with support of CDC and PMI; data not shared so far but CDC confirmed presence of R561H.
Article:


Summary:

• Patients (N = 148) in five districts of northwest Ethiopia were enrolled in a 28-day AL TES.
• A unique *Pfkelch 13* mutation (R622I) was identified in 3/125 (2.4%) samples.
• The 3 isolates with R622I were from Negade-Bahir and Aykel districts in Amhara region close to the Ethiopia-Sudan border.
• One of three patients with the mutant strain was day3+; however, all patients cleared parasites by day 28.
DHA-piperaquine was considered in the WHO treatment guideline when Duo-cotexin® was registered in China;

Eurartesim® was approved later by EMA and was also pre-qualified by WHO;

PQ department indicates on its website that this ACT is unstable > 30°C and 75% humidity (tropical conditions);

DHA is thermally and chemically labile (> artesunate > artemether) (≠ piperaquine)
  • temperature, humidity and contact with partner medicine (Haynes et al., Chem Med Chem, 2007);

So far no other combination is pre-qualified;

Eurartesim® is difficult to procure, which leaves the door open to many generic compounds of various quality in Africa.
Prevalence of *Pfplasmepsin* 2-3 increased copy number in some African countries

<table>
<thead>
<tr>
<th>Year</th>
<th>Countries</th>
<th>Prevalence</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012-14</td>
<td>Mali</td>
<td>7/65 (10.8%)</td>
<td>TES</td>
</tr>
<tr>
<td>2013</td>
<td>Comoros</td>
<td>3/46 (6.5%)</td>
<td>TES</td>
</tr>
<tr>
<td>2013-15</td>
<td>Rwanda</td>
<td>4/130 (3.1%)</td>
<td>TES</td>
</tr>
<tr>
<td>2015</td>
<td>Mozambique</td>
<td>0/87 (0%)</td>
<td>TES</td>
</tr>
<tr>
<td>2015</td>
<td>Mozambique</td>
<td>1/88 (1.1%)</td>
<td>TES</td>
</tr>
<tr>
<td>2015</td>
<td>Mozambique</td>
<td>1/89 (1.1%)</td>
<td>TES</td>
</tr>
<tr>
<td>2015</td>
<td>Mozambique</td>
<td>2/87 (2.3%)</td>
<td>TES</td>
</tr>
<tr>
<td>2015</td>
<td>Mozambique</td>
<td>3/61 (4.9%)</td>
<td>Pre-MDA</td>
</tr>
<tr>
<td>2016</td>
<td>Mozambique</td>
<td>1/19 (5.3%)</td>
<td>Post-MDA</td>
</tr>
<tr>
<td>2017</td>
<td>Eritrea</td>
<td>8/42 (19.0%)</td>
<td>TES</td>
</tr>
</tbody>
</table>

Presence of multicopy *Pfplasmepsin* 2-3 in Africa is a potential concern in particular with the massive of the uncontrolled use of DHA-PIP of various quality.
WHO has a normative and public health role:

- How can WHO retrieve data from countries or research institutes refusing to share these data when there is a public health concern?
- Huge delays between evidence generation and publication.

Several different methodologies are used to assess the origin of a parasite:

- What would be the minimum/optimal information to confirm the origin of a resistant parasite?

Use substandard DHA-PIP (mainly as a monotherapy) could pose a public health problem for Africa

- Urgent need to support/conduct QC of the multiple generic DHA-PIP compounds circulating in Africa.
Thank you for your attention