Antimalarial drug resistance.

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Centre for Medical Parasitology
ISIM, University of Copenhagen

Building Stronger Universities course

*The use of next generation sequencing and bioinformatics for malaria research – the example of drug resistance*
Presentation

• The use of drugs in treatment and control of malaria – historical perspective

• The development/evolution of drug resistance – historical perspective

• Drug resistance – evolution of drug resistance on a molecular level

• IPTp/SMC and MDA, risk of drug resistance selection
Malaria has been here for some time..

Ex. Greece:

Alexander the Great (died 323 BC)

• May 29\textsuperscript{th}: He slept in the bathroom because he was feverish.
• June 1\textsuperscript{st}: The fever grew more intense
• June 5\textsuperscript{th}: Was moved to another palace; slept a little, high fever
• June 6\textsuperscript{th}: High fever and speechless
• June 10\textsuperscript{th}: Toward evening, he died

Reason? Malaria or typhoid fever or?

Ancestry and Pathology in King Tutankhamun’s Family

These results suggest avascular bone necrosis in conjunction with the malarial infection as the most likely cause of death in Tutankhamun.
Malaria has been here for some time..

“Medicines” have always been used against malaria

**Quinine**
Bark of a tree native to South America.

Legend: first brought to Europe by a Countess who had been treated with it in Peru in the 1600s.

The bark was named cinchona in 1742 by Linnaeus
Artemisinins (In Chinese: qinghao)

A Handbook of Prescriptions for Emergencies

Ge Hong (year 284–346)

For relieving “malaria” symptoms:

“A handful of qinghao immersed with 2 liters of water, wring out the juice and drink it all”
Main malaria control strategies

Mosquito control:
• Indoor residual spraying (IRS)

Mosquito-human contact prevention
• Insecticide treated nets (ITNs)

Rapid diagnosis & appropriate treatment
• Use of Rapid Diagnostic Tests

• Treatment
Artemisinin-based combination therapies (ACTs)
• Prophylactic treatment:
  Intermittent preventive treatment
  In pregnancy (IPTp)
  In children (IPTc/SMC)
Malaria control – efficacious treatment

2001: WHO recommended artemisinin-based combination therapies (ACTs)

ACTs: a combination of an artemisinin and another drug:
- Artemisinins kills parasites quickly (1-3 days)
- Partner drug kills the remaining parasites and protects
Intermittent preventive treatment of vulnerable groups

Pregnancy (IPTp) with sulfadoxine-pyrimethamine (SP)
- IPTp – SP: All pregnant women at each scheduled antenatal care visit until the time of delivery

Seasonal Malaria Chemoprevention (SMC) of children <5 years
- Treatment amodiaquine + SP given at monthly intervals to children in areas of highly seasonal transmission

IPTi...
History of antimalarial drugs, development, drug resistance

- CQ (1940)
- PGR (1945)
- PyrR (1950)
- QR (1955)
- Art (1960)
- Mef (1965)
- MefR (1970)
- Ato (1975)
- AtoR (1980)
- Ato–PGR (1985)
- CQR (S.E. Asia, S. America) (1940–1950)
- Pyr–SDX (1950–1960)
- Pyr–SDXR (1960–1970)
Drug resistance will always evolve
Drug resistance emerges in SE Asia and spreads to Africa

- Developed in the 1940’s
- Used large-scale after 1945
- CQ-R 1957

- (Pyr, developed in 1952)
- Developed in the 1967 (SP)
- SP-R, in 1967

- Developed in the 1970-80’s
- WHO recommendation, 2001

Impact of drug resistance on burden of malaria

Malaria control strategies – drug(s) resistance

Why always the similar SEA origin of resistance development?
Development of resistance

Drug action:
E.g. inhibits enzyme(s) => death

Drug resistance:
Enzyme(s) have changed => Drug is unable to inhibit the enzymes => survival

The cause of change:
SNPs causes changes in essential enzymes that drugs inhibit

Prevalence of mutations in certain genes of malaria parasites => level of drug resistance
Changes causing drug resistance

CQ resistance

<table>
<thead>
<tr>
<th>Drug</th>
<th>Resistance gene</th>
<th>Main SNPs (aa at position)</th>
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<tr>
<td>Chloroquine</td>
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<td>72-76 SVMN'T (MT)</td>
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</table>
Development of drug resistance – observations *in vivo*

**Table 1** Chloroquine efficacy, EANMAT area, in patients aged 6–59 months, 1998–2000

<table>
<thead>
<tr>
<th>Sentinel site</th>
<th>Date</th>
<th>Number of patients</th>
<th>ACR %</th>
<th>ETF %</th>
<th>LTF %</th>
<th>Total clinical failure rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kenya/Busia</td>
<td>February 1999</td>
<td>44</td>
<td>36</td>
<td>5</td>
<td>59</td>
<td>64</td>
</tr>
<tr>
<td>Tanzania/Masasi</td>
<td>March 1999</td>
<td>62</td>
<td>69</td>
<td>22</td>
<td>9</td>
<td>31</td>
</tr>
<tr>
<td>Tanzania/Mlimba</td>
<td>January 1999</td>
<td>71</td>
<td>29</td>
<td>32</td>
<td>39</td>
<td>71</td>
</tr>
<tr>
<td>Uganda/Apac</td>
<td>June 1999</td>
<td>55</td>
<td>80</td>
<td>13</td>
<td>7</td>
<td>20</td>
</tr>
<tr>
<td>Uganda/Arua</td>
<td>June 1998</td>
<td>60</td>
<td>77</td>
<td>10</td>
<td>13</td>
<td>23</td>
</tr>
<tr>
<td>Uganda/Jinja</td>
<td>June 1998</td>
<td>22</td>
<td>68</td>
<td>23</td>
<td>0</td>
<td>23</td>
</tr>
<tr>
<td>Uganda/Kabarole</td>
<td>June 1999</td>
<td>16</td>
<td>44</td>
<td>25</td>
<td>31</td>
<td>56</td>
</tr>
<tr>
<td>Uganda/Rukungiri</td>
<td>June 1999</td>
<td>49</td>
<td>90</td>
<td>10</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Uganda/Tororo</td>
<td>June 1999</td>
<td>62</td>
<td>55</td>
<td>18</td>
<td>27</td>
<td>45</td>
</tr>
<tr>
<td>Rwanda/Nyarurema</td>
<td>October 1999</td>
<td>59</td>
<td>42</td>
<td>46</td>
<td>12</td>
<td>58</td>
</tr>
<tr>
<td>Rwanda/Rwaza</td>
<td>October 1999</td>
<td>54</td>
<td>46</td>
<td>13</td>
<td>41</td>
<td>54</td>
</tr>
<tr>
<td>Rwanda/Kivumu</td>
<td>February 2000</td>
<td>54</td>
<td>81</td>
<td>15</td>
<td>4</td>
<td>19</td>
</tr>
<tr>
<td>Zanzibar/Kivunge</td>
<td>April 2000</td>
<td>74</td>
<td>39</td>
<td>47</td>
<td>14</td>
<td>61</td>
</tr>
<tr>
<td>Zanzibar/Micheweni</td>
<td>April 2000</td>
<td>78</td>
<td>40</td>
<td>35</td>
<td>25</td>
<td>60</td>
</tr>
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EANMAT, TMIH volume 8 no 10 pp 860–867 2003
Development of drug resistance – molecular observations

Tanzania; CQ was abandoned in 2001 (officially)

- Tanga, NE-Tanzania
- >95% prev. Of CQ resistant parasites

(Unpublished data)

When CQ is not in use anymore...
Temporal changes of drug resistance – molecular observations

- Samples from children living in two villages of Tanga region

Molecular surveillance of drug resistance

<table>
<thead>
<tr>
<th>Tanzania, region</th>
<th>Prevalence of CVMNK</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
</tr>
<tr>
<td>Tanga</td>
<td>116</td>
</tr>
<tr>
<td>Coastal</td>
<td>139</td>
</tr>
<tr>
<td>Mtwara</td>
<td>71</td>
</tr>
<tr>
<td>Kagera</td>
<td>97</td>
</tr>
<tr>
<td>Mwanza</td>
<td>171</td>
</tr>
<tr>
<td>Mbeya</td>
<td>147</td>
</tr>
<tr>
<td>Overall</td>
<td>741</td>
</tr>
</tbody>
</table>

After Mohamed et al. Malaria J. (2013)
Country-wide Surveillance of CQ resistance in Senegal

CQ-R

Mutant Pfcrt (CVIET)
Wildtype (CVMNK)

Sub-sample:
- 50 RDTs/district

Changes causing drug resistance (SP)

SP resistance

Sulfadoxine

GTP → Dihydropteroate synthetase (DHPS) → Dihydropteroate → Dihydrofolate synthetase

PABA

Dihydrofolate reductase (DHFR)

Tetrahydrofolate → Pyrimethamine

Thymidylate synthetase

dUTP → dTMP → DNA

Folate synthesis
## Changes causing drug resistance (SP)

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<tr>
<td>Sulfadoxine</td>
<td><em>Pfdhps</em></td>
<td>A437G, K540E</td>
<td>High SP resistance</td>
</tr>
<tr>
<td></td>
<td></td>
<td>437+540 =&gt; 2 MT (+ <em>Pfdhfr</em> =&gt; 5 MT)</td>
<td></td>
</tr>
</tbody>
</table>

**Diagram:**

- **Pfdhfr**
  - 51
  - 59
  - 108

- **Pfdhps**
  - 437
  - 540
Changes causing drug resistance in vivo (SP)

Gray bars: sensitive. Black bars: TF

3MT in Pfdhfr + 2 MT in Pfdhps = ↑ risk of TF

Kublin et al. JID 2002;185 (1st feb.)
Monitoring drug resistance – *in vivo*

Not always clear-cut relationship between “full house” mutations and TF
Acquisition of immunity

Figure 1: Relation between age and malaria severity in an area of moderate transmission intensity. With repeated exposure protection is acquired, first against severe malaria, then against illness with malaria, and, much more slowly, against microscopy-detectable parasitaemia.³

White et al. Lancet 2014 (383)

Related to transmission: areas of high transmission. Impact of immunity on treatment outcome...?
Other factors that impact treatment outcome...

Table 2: Frequency of \textit{P. falciparum} polymorphisms in \textit{dhfr}, \textit{dhps} and \textit{crt} genes in relation to treatment outcome

<table>
<thead>
<tr>
<th>Haplotypes</th>
<th>TF</th>
<th>ACPR</th>
</tr>
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<tbody>
<tr>
<td>SP</td>
<td>34</td>
<td>16</td>
</tr>
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</table>

\textit{dhfr}
- wildtype
- S108N, single
- C59R + S108N, double
- N511 + S108N, double
- N511 + C59R + S108N, triple
- Mixed*  

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<tr>
<th>Haplotypes</th>
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<th>ACPR</th>
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| \textit{dhps}
- wildtype
- A437G, single
- A437G + K540E, double
- single-double combined
- Mixed*  

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<th>ACPR</th>
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</table>
| \textit{dhfr-cdhps}
- triple-double
- triple-single
- double-double  

- Small clinical trial
- Chamwino village, Dodoma region, Tanzania
- Children, 6-59months
- Both SP and AQ
- Baseline prev of 5MT

Enevold et al. Malaria Journal 2007, 6:153
Other factors that impact treatment outcome...immunity

Thus, some level of acquired immunity contribute to ACPR (implications for elimination efforts?)
Other factors?
Changes causing drug resistance – observations *in vivo* (SP)

- The proportion of sites in the ‘alert’ phase increased from 5/17 (29%) pre-2000 to 13/32 (41%) post-2000

- The proportion of sites where treatment failure exceeded 25% increased from 1/17 (6%) pre-2000 to 7/32 (22%) post-2000

- WHO recommends ACTs (2001)
SP is still in use for IPT of vulnerable groups

Infants: IPTi with sulfadoxine-pyrimethamine (SP)

Pregnancy (IPTp) with SP
- IPTp – SP: All pregnant women at each scheduled antenatal care visit until the time of delivery

Seasonal Malaria Chemoprevention (SMC) of children <5 years
- Treatment amodiaquine + SP given at monthly intervals to children in areas of highly seasonal transmission
SP for IPTi

SP is still recommended for IPTi (as policy: only Burkina Faso)

- IPTi: provide effective protection against multiple infections and malaria related morbidity  

- However, Tanzania (with high level of SP resistance), no protective effect  

- IPTi: effective protection when 3MT in *Pfdhfr* and 347 MT in *Pfdhps*

- However, + 540 MT...
Changes causing drug resistance – molecular observations

2004–2008 (squares)
2009–2013 (circles)

SP for IPTi

• Protective effectiveness of IPTi seemed only to be compromised in areas of high 5MT prevalence (and thus high 540E) – not in areas with “moderate” prevalence

• Threshold for when to use IPTi; based on molecular data:
  • WHO recommend *only* to use SP for IPTi

  "...where parasite resistance to SP is not high – defined as a prevalence of the PfDHPS 540 mutation of ≤ 50%.”

http://www.who.int/malaria/news/WHO_policy_recommendation_IPTi_032010.pdf?ua=1
Threshold for protective efficacy of IPTi

Pfdhps- 540E

www.drugresistancemaps.org
Molecular observations

SP Molecular Surveyor

http://www.wwarn.org/dhfr-dhps-surveyor/#0
SP is still in use for IPT of vulnerable groups

SP is used as IPT for pregnant women (IPTp)

- IPTp seems effective even in areas with high prev. of 540 MT;
  however, what if an additional mutation emerged?

(5MT + 1 extra MT..., e.g. 540E + 581G)
Prevalence of *Pfdhps* haplotypes in Korogwe, Tanzania –


Is IPTp still effective in areas where we now have the additional MT? (now; 6MT in *Pfdhfr* and *Pfdhps*)
SP is still in use for IPT of vulnerable groups

STOPPAM – Strategies to Prevent Pregnancy Associated Malaria

• Cohort of pregnant women living in Korogwe, NE-Tanzania
• Sep. 2008 – Oct. 2010

• 995 women included, all on IPTp
• Low prevalence of malaria; Among the women completing follow-up, 76 had a total of 96 episodes of malaria.

• Prevalence of 540 MT = 100%, 581 MT = 54%

SP is still in use for IPT of vulnerable groups

2MT vs. 3MT infections => mean reduction of birthweight of 326g

Changes causing drug resistance – molecular observations


2004–2008 (squares)
2009–2013 (circles)
Fig. 1 Regional sites and distribution of *Pfhrp3* polymorphisms in Tanzania. a *Pfhrp3 K540E* and b *Pfhrp3 A581G*. Mutants are shown in red and wild types in green. Mixed genotypes are shown in light blue. All samples were collected in 2010/2011 except data shown in purple. Shown in purple is preliminary data for samples collected in 2014 for Mwanza and Ruvuma regions where similarity in *Pfhrp3* 540E and 581G prevalence between Ruvuma and neighbouring Mtwara region is observed.
Alternatives to SP for IPTp

EU-EDCTP funded IMPROVE study
SP vs. dihydro-artemisinin-piperaquine +/- azithromycin

- Sites in Kenya, Malawi and Tanzania
- 4680 women
- Treated and followed 2\textsuperscript{nd} and 3\textsuperscript{rd} trimester
- Molecular markers of drug resistance
Changes causing drug resistance – molecular observations

- Seems to be no challenges in West Africa
- Only few *Pfdhps* 540E and no 581G..
- Other mutations in *Pfdhfr* and/or *Pfdhps*?

“The distribution of the *Pfdhps*-431V mutation was widespread throughout Nigeria with the highest prevalence in Enugu (46%).”

“Based on our findings, it has become crucial to evaluate the impact of dhps-VAGKGS and other combinations of 431V in SMC and IPTp since this emerging mutation is on the increase.”

Malaria control strategies – drug(s) resistance

How do we know the actual route of resistance spread?
The route of resistance (SP)

Do the development and spread of resistance

1. Arise independently at several distinct locations?

=> Mutations may arise independently in each patient. (Infections are in the order of $10^{10}$-$10^{12}$ parasites/person). Key mutations in Pfdhfr can occur as $2.5\times10^{-9}$/parasite replication

OR

2. Arise locally and gene flow is the reason for the spread?
The route of resistance (SP)

**Microsatellite analysis**: minute polymorphisms in sequences of flanking regions surrounding *Pfdhfr* and *Pfdhps*:

When a beneficial mutation spreads through a population, flanking **neutral** polymorphisms are carried along (“hitchhiking”)

=> removal of genetic variation at the sites surrounding the selection

If polymorphisms in the flanking regions is limited for resistant parasites at different localities

=> a selective sweep has driven the spread of resistance
The route of resistance (SP)

- **Triple mutants**
- **Double mutants**
- **Single mutants**
- **Sensitives**

**Green** locus 0.5kb from DHFR
**Blue** locus 4.5kb from DHFR
**Yellow** locus 17kb from DHFR

Roper et al. Lancet 2003; 361:1174-81
The route of resistance (SP)

Samples from various sites in South Africa and Tanzania

Triple dhfr-MT had all the same microsatellite haplotype:
Both SA and TZ samples

Roper et al. Lancet 2003; 361:1174-81
The route of resistance (SP)

Markers flanking resistant dhfr alleles: extremely reduced diversity....from five SE Asian countries

Malaria ACT drug resistance


![Distribution of malarial multidrug resistance in 2016](image-url)

*Figure 4.8 Distribution of malarial multidrug resistance 2016. Source: WHO database*

AFRICA

*When...?*
## Molecular markers of drug resistance - artemisinins

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<td></td>
<td>+581=&gt; 3 MT</td>
<td>Super SP resistance</td>
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<tr>
<td>Artemisinins</td>
<td><em>K13</em></td>
<td>Various</td>
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Artemisinin resistance marker, K13

Artemisinin resistance marker, K13

Menard et al. NEMJ 374;25 June 23, 2016
Artemisinin resistance marker, K13

Menard et al. NEMJ 374;25 June 23, 2016
Parasites from >1100 African infections collected since 2002 from 14 sites across SSA

- Not same SEA mutations in parasite populations associated with artemisinin resistance
- 15 coding mutations, including 12 novel mutations, consistent with a large reservoir of naturally occurring K13-propeller variation.

Taylor et al. JID 2015:211 (1 March)

K13 is a marker of resistance before TF are observed...
Molecular markers of drug resistance - *Pfmdr*

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<td>C59R</td>
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<td>A581G</td>
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</tr>
<tr>
<td>Various</td>
<td><em>Pfmdr1</em></td>
<td>N86Y, Y184F, D1246Y</td>
<td></td>
</tr>
</tbody>
</table>
Molecular markers of drug resistance – Pfmdr1

Relevant resistance marker for several drugs, including ACTs:

A meta-analysis estimated that parasites with the N86 wildtype genotype are 4.7 times more likely to recrudesce after AL treatment than parasites with the 86Y genotype


In general:
AQ alone as well as AS-AQ has been shown to select for Pfmdr1 86Y, Y184 and 1246Y (the YYY haplotype) while the AL combination selects for N86, 184F and D1246 (the NFD haplotype)

Otienoburu et al. Malar J 2016;15:452
Molecular markers of drug resistance – *Pfmdr1*

- Mainly, 3 policies of ACT implementation in SSA:
  - AL
  - AS-AQ
  - AL and AS/AQ

- Analyse the effect of implementation of these strategies on the temporal trends in the population prevalence of SNPs in *Pfmdr1* (86, 184, 1246) across Africa (Systematic review of published *Pfmdr1* prev. data)

  - Dividing countries into AL, AS/AQ or AL & AS/AQ policies

  - Literature search: 930 articles, 171 met the inclusion criteria
  - Data from 397 surveys measuring the prevalence or frequency of at least one *Pfmdr1* polymorphism in 30 countries

*Okell et al. BMJ Global Health (accepted)*
Molecular markers of drug resistance – Pfmdr1

Okell et al. BMJ Global Health (accepted)
Molecular markers of drug resistance – *Pfmdr1*

- 86Y-decline was fastest in AL countries (A vs. B and C).
- Higher initial 86Y freq. => a more rapid decline over time
- Overall, 184F increased in freq., highest for AL countries
- Selection of 1246D, mainly in AL countries
Molecular markers of drug resistance – *Pfmdr1*

- Widespread decline in the prevalence of the 86Y and 1246Y mutations (increase of N86 and D1246), particularly in AL countries, suggesting slightly increased sensitivity to AS-AQ and slightly reduced sensitivity to AL

Implications?

Design of drug policies which exploit the collateral sensitivity of these two drugs, such as drug cycling, sequential ACT treatments or multiple first line therapies.
Summary

• Antimalarial drug resistance will always evolve

• Molecular markers are becoming increasingly important for the surveillance of drug resistance (and for drug policy decisions)

• We need to use available ACTs wisely (and innovatively)
  • We need new (non ACT) antimalarials!

• No major problems with ACT resistance as yet in SSA
  • No significant selection of resistance (of public health relevance by SMC/IPT)

• Elimination: keep a eye on drug resistance when attempting to eliminate malaria using drugs (MDA) ...and as well for control by IPT/SMC strategies
Malaria – Tanzania – prevalence of malaria in <20 years

Year

Pf prevalence (%)

0 20 40 60 80 100

Mobile clinic Magoda
Mobile clinic Mpapayu
ITNs Magoda
ITNs Mpapayu
Dispensary built
ACTs deployed

Ishengoma Malaria J 12(338): 2013
Impact of vector control...

Masaika village

- Less rains each year
- Less mosquitoes (two species)

Meyrowitsch et al. Malaria J. 2011 (10:188)
Impact of vector control...?

Kirare village

Not less rains (chaotic)
Less mosquitoes
No major control (ITNs : 27%)
Ishengoma et al. MJ 12(338): 2013 (and unpublished)

Dispensary (2005)
Are preventive treatments selecting for drug resistance?

Are SMC strategies selecting for resistance?

Effectiveness of Seasonal Malaria Chemoprevention in Children under Ten Years of Age in Senegal: A Stepped-Wedge Cluster-Randomised Trial

- Senegal, <10years
- Overall prevalence of pf dhfr, pf dhps, pf crt, and pf md r mutations was compared: lower in SMC areas vs. ctrls.

Measuring the impact of seasonal malaria chemoprevention as part of routine malaria control in Kita, Mali

- Mali, <5years
- The frequencies MTs in pf dhfr, pf dhps, Pf crt and Pf md r1 were similar between SMC vs. ctrl before and after the intervention, except for Pf dhps A437G, which was higher in the SMC
Elimination (and control) – approaches using drugs (MDA)

MDA: previous attempts (direct MDA)

<table>
<thead>
<tr>
<th>Year</th>
<th>Location</th>
<th>Drug</th>
<th>Insecticide</th>
<th>Population</th>
<th>Coverage (%)</th>
<th>Reported outcome</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1931</td>
<td>Liberia</td>
<td>Plasmoquine</td>
<td>–</td>
<td>124</td>
<td>100</td>
<td>Reduction in mosquito infection rate and in gametocyte carriers. No follow-up data available.</td>
<td>[1]</td>
</tr>
<tr>
<td>1948</td>
<td>Kericho, Kenya,</td>
<td>Proguanil</td>
<td>DDT</td>
<td>10 000</td>
<td>NK</td>
<td>Drop in malaria incidence from 56 per 1000 people per month before 1948 to 5 per 1000 people per month in 1949.</td>
<td>[8]</td>
</tr>
<tr>
<td>1953–54</td>
<td>Nandi Hills, Kenya</td>
<td>PYR</td>
<td>–</td>
<td>83 000</td>
<td>95</td>
<td>Reduction in parasite prevalence from 23% to 2.3%.</td>
<td>[10,11]</td>
</tr>
<tr>
<td>1959–60</td>
<td>Kigezi Highlands, Uganda</td>
<td>CQ, PYR</td>
<td>DDT</td>
<td>16 000</td>
<td>50</td>
<td>Eradication of the vector and rapid elimination of malaria for an unspecified period.</td>
<td>[12,13]</td>
</tr>
<tr>
<td>1960</td>
<td>Maroua, Cameroon</td>
<td>CQ + PYR</td>
<td>DDT</td>
<td>67 500</td>
<td>76–92</td>
<td>Decrease in parasite prevalence, transmission was not interrupted.</td>
<td>[15]</td>
</tr>
<tr>
<td>1960–61</td>
<td>Bobo-Dioulasso, Upper Volta</td>
<td>CQ + PMQ, or amodiaquine</td>
<td>DDT</td>
<td>13 340</td>
<td>&gt;80</td>
<td>Decrease in numbers of parasites, gametocytes, and sporozoites, but no interruption of malaria transmission.</td>
<td>[14]</td>
</tr>
<tr>
<td>1960–61</td>
<td>Andhra Pradesh, India</td>
<td>CQ + Daraprim</td>
<td>DDT</td>
<td>35 000</td>
<td>NK</td>
<td>Interruption of transmission for an unspecified period.</td>
<td>[23]</td>
</tr>
<tr>
<td>1962–ongoing</td>
<td>Southern provinces, China</td>
<td>CQ or piperazine or SD + PMQ</td>
<td>Residual insecticides</td>
<td>&gt;30 000 000</td>
<td>NK</td>
<td>Reduction in reported malaria cases from 6 970 000 in 1955 to 24 688 in 2000.</td>
<td>[32,33,58,59]</td>
</tr>
<tr>
<td>1962–64</td>
<td>Uttar Pradesh, India</td>
<td>PMQ</td>
<td>DDT</td>
<td>7248</td>
<td>72</td>
<td>Malaria eradication for an unspecified period.</td>
<td>[24]</td>
</tr>
</tbody>
</table>

Elimination (and control) – approaches using drugs (MDA)

Table 1. An overview of direct mass drug administration projects

<table>
<thead>
<tr>
<th>Year</th>
<th>Location</th>
<th>Drug</th>
<th>Insecticide</th>
<th>Population</th>
<th>Coverage (%)</th>
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<th>Refs</th>
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<td>100</td>
<td>Reduction in mosquito infection rate and in gametocyte carriers. No follow-up data available.</td>
<td>[1]</td>
</tr>
<tr>
<td>1984–1985</td>
<td>Sabah, Malaysia</td>
<td>PYR + SD, PMQ</td>
<td>Permethrin-impregnated bednets</td>
<td>5 villages</td>
<td>Low</td>
<td>Reduction in parasite prevalence from 46% to 11% for two months.</td>
<td>[28]</td>
</tr>
<tr>
<td>1987–1989</td>
<td>Sumatra, Indonesia</td>
<td>PYR + SD, PMQ</td>
<td>–</td>
<td>3 villages</td>
<td>100%</td>
<td>P. falciparum prevalence decreased from 14% to 1% eight months after intervention.</td>
<td>[29]</td>
</tr>
<tr>
<td>1991</td>
<td>Anetuyum, Vanuatu</td>
<td>PYR + SD, PMQ</td>
<td>Permethrin-impregnated bednets</td>
<td>700</td>
<td>&gt;88%</td>
<td>Malaria eradicated from the island during seven years of follow up.</td>
<td>[31]</td>
</tr>
<tr>
<td>1999</td>
<td>Farefenni, The Gambia</td>
<td>PYR + SD, artesunate</td>
<td>–</td>
<td>16 400</td>
<td>85%</td>
<td>During two months following MDA, there was a lower malaria incidence in children from treated villages.</td>
<td>[20]</td>
</tr>
</tbody>
</table>

*aAbbreviations: CQ, chloroquine; NK, not known; PYR, pyrimethamine; PMQ, primaquine; SD, sulfadoxine.*

Initially...incidence rate in treated villages was significantly lower vs. ctrl. In subsequent months, the incidence was slightly higher in the MDA villages. Overall, no benefit of the MDA was detected over the course of the malaria transmission season.

In general, no apparent selection of drug resistance using direct MDA

Elimination (and control) – approaches using drugs (MDA)

MDA: previous attempts (indirect MDA)

Table 3. Mass administration through medicated salts using Pinotti's methoda

<table>
<thead>
<tr>
<th>Year</th>
<th>Location</th>
<th>Drug</th>
<th>Insecticide</th>
<th>Population</th>
<th>Coverage</th>
<th>Reported Outcome</th>
</tr>
</thead>
</table>
| 1952–1953 | Para state, Brazil         | CQ          | None        | 900        | NK       | NK                                               | [7]  
| 1952–1954 | Para state, Brazil         | CQ          | None        | 173        | NK       | NK                                               | [7]  
| 1953–1954 | Maranhao state, Brazil     | CQ          | None        | 139        | NK       | NK                                               | [7]  
| 1956–?    | Minas Gerais state, Brazil | CQ          | None        | 5640       | NK       | NK                                               | [7]  
| 1957–?    | Sta Catarina state, Brazil | CQ          | None        | 20 000     | NK       | NK                                               | [7]  
| 1957–?    | Amapa state, Brazil        | CQ          | None        | NK         | NK       | NK                                               | [7]  
| 1959–?    | Amazon state, Brazil       | CQ          | None        | 2,500,000  | NK       | NK                                               | [7]  
| 1958–1961 | West New Guinea (later Irian Jaya) | Initially PYR, Later CQ | DDT | 10 000 | NK | Reduction in *P. falciparum* prevalence. PYR resistance was observed within 3 months | [39]  
| 1958–?    | Sarawak, Akah and Sitat River | NK          | NK          | 98         | NK       | NK                                               | [7]  
| 1958–?    | Angola                     | CQ          | NK          | 227        | NK       | NK                                               | [7]  
| 1959–1967 | Iran, Kazeroun area        | PYR, CQ, amodiaquine | NK | 18 050 | NK | Drug resistance                                 | [5,6,41]  
| 1960–1965 | Kampuchea and Cambodia     | Initially PYR and later CQ | - | 20 000 | NK | Reduction in *P. falciparum* prevalence. CQ resistance was observed within months. | [40]  
| 1961–1965 | Guyana                     | CQ          | DDT         | 48 500     | NK       | Reduction in *P. falciparum* prevalence. CQ resistance was observed within months. | [7]  
| 1961–1978 | Mto Wa Mbo, Tanzania       | CQ, amodiaquine | NK | 12 393 | NK | NK                                               | [7]  
| 1962–?    | Mindoro, Philippines       | NK          | NK          | NK         | NK       | NK                                               | [7]  

*aAbbreviations: CQ, chloroquine; PYR, pyrimethamine; NK, not known."
Elimination (and control) – approaches using drugs (MDA)

Newer MDA studies (and possible selection of drug resistance)

- Artemisinin–piperaquine (AP) +/- primaquine
- 3 monthly rounds across Anjouan Island, Union of Comoros.
- Coverage: 85 to 93% in: 2 districts (AP+PM, pop: 97.164) 5 districts (AP alone, 224.471)
- Between the months of April–September in both 2012 and 2013, average monthly malaria hospital rates/100.000:
  - 310.8 to 2.06 (AP+PM) \( P = .00007 \)
  - 412.1 to 2.60 (AP) \( P < .00001 \)

MDA selection of resistance

Table 2. *P. falciparum K13* Sequence Polymorphisms on Anjouan Island

<table>
<thead>
<tr>
<th>Mutations</th>
<th>Amino Acid Change and Positionª</th>
<th>Genetic Changeª</th>
<th>Number of Changes Detected (%)³ Before MDA (n = 196)ª</th>
<th>After MDA (n = 52)ª</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synonymous</td>
<td>Y500Y</td>
<td>TAT→TAC</td>
<td>2 (1.0)</td>
<td>2 (3.8)</td>
</tr>
<tr>
<td></td>
<td>N531N</td>
<td>AAT→AAC</td>
<td>3 (1.5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Non-synonymous</td>
<td>D464H</td>
<td>GAT→CAT</td>
<td>5 (2.6)</td>
<td>2 (3.8)</td>
</tr>
<tr>
<td></td>
<td>S477Y</td>
<td>TCT→TAT</td>
<td>0 (0)</td>
<td>1 (1.9)</td>
</tr>
<tr>
<td></td>
<td>N490H</td>
<td>AAT→CAT</td>
<td>6 (3.1)</td>
<td>1 (1.9)</td>
</tr>
<tr>
<td></td>
<td>V520A</td>
<td>GTT→GCT</td>
<td>0 (0)</td>
<td>1 (1.9)</td>
</tr>
<tr>
<td></td>
<td>N564H</td>
<td>AAT→CAT</td>
<td>3 (1.5)</td>
<td>1 (1.9)</td>
</tr>
<tr>
<td></td>
<td>A578S</td>
<td>GCT→TCT</td>
<td>2 (1.0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

- Analysis of 52 malaria samples after MDA showed no evidence for selection of PfK13 Kelch-propeller mutations

Elimination (and control) – approaches using drugs (MDA)

- 4 villages, western Cambodia, 3 rounds of MDA (a 3-day course of DHA+pip)
- Coverage: all 3 rounds: 58%
- \( P. \ falciparum \) incidence: lower in intervention villages: 1.5/1000/year vs. 37.1/1000/year (\( P= .002 \)).
- Following MDA in 2016, there were no clinical falciparum malaria cases over 12 months

Tripura et al. CID 2018:67 (15 September)
MDA selection of resistance

• 27 (48%), K13 sequenced: All were positive for the C580Y haplotype. None was positive for other K13 markers....selection?

• Among 35 *falciparum* malaria cases detected up to April 2016, all had K13 MT: 34 had the K13 C580Y mutation and 1 had the K13 F446I mutation.

*Tripura et al. CID 2018:67 (15 September)* •
Elimination (and control) – approaches using drugs (MDA)

- Myanmar
- MDA, 3-day DHA-PIP + single low-dose primaquine, adm. monthly for three months

- 3 months after starting MDA, prevalence was lower in intervention villages; 0.4% versus 2.7%, p=0.0014

- After nine months the difference was no longer significant: 2.0% versus 0.9%, p=0.10.

Landier et al. Wellcome Open Res. 2017 Sep 6;2:81
MDA selection of resistance

Table 4. Detection of molecular markers of antimalarial resistance in *Plasmodium falciparum* positive samples according to period before and after mass drug administration (MDA).

<table>
<thead>
<tr>
<th></th>
<th>Before MDA</th>
<th>After MDA</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>PF positive samples by uPCR</td>
<td>191</td>
<td>56</td>
<td>247</td>
</tr>
<tr>
<td>Sample with PfK13 result</td>
<td>77 (40.3%)</td>
<td>30 (53.6%)</td>
<td>107 (43.3%)</td>
</tr>
<tr>
<td>Samples with PfK13 mutation</td>
<td>66</td>
<td>17</td>
<td>83</td>
</tr>
<tr>
<td>PfK13 Mutation prevalence [95% CI]</td>
<td>85.6% [75.9–92.6]</td>
<td>56.7% [37.4–74.5]</td>
<td>77.6% [68.5–85.1]</td>
</tr>
<tr>
<td>Sample with Piperaquine resistance result</td>
<td>53 (27.7%)</td>
<td>16 (28.6%)</td>
<td>69 (27.9%)</td>
</tr>
<tr>
<td>Samples with Piperaquine resistance marker</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Piperaquine resistance prevalence [95% CI]</td>
<td>0% [0–6.7]</td>
<td>0% [0–20.6]</td>
<td>0% [0–5.2]</td>
</tr>
</tbody>
</table>

Landier et al. Wellcome Open Res. 2017 Sep 6;2:81

- The prevalence of K13 mutants was 85.6% before and 56.7% after MDA, thus no selection
- Most frequent K13 mutation was C580Y (37.4%)
- No markers of piperaquine resistance were found either (plasmepsin 2 amplification)
MDA selection of resistance – last!

- Magoda village, Tanga Region, Northeastern Tanzania
- May 1993 to May 1994
- Weekly Maloprim (dapsone-pyrimethamine) to children 1-9 years (n=249)
- Impact on clinical malaria, splenomegaly etc.
- 52 weeks of follow-up

MDA selection of resistance

Table 5. Comparison of incidence of clinical malaria\textsuperscript{a} in children aged 1–4 years receiving Maloprim\textsuperscript{®} or placebo

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Maloprim\textsuperscript{®}</th>
<th>( p )\textsuperscript{b}</th>
<th>Placebo</th>
<th>Relative rate\textsuperscript{c}</th>
<th>Maloprim\textsuperscript{®} protective efficacy\textsuperscript{c,d}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–24</td>
<td>0.88 (n=23)</td>
<td>&lt;0.0001</td>
<td>2.70 (n=71)</td>
<td>0.33 (0.21, 0.53)</td>
<td>67% (47%, 79%)</td>
</tr>
<tr>
<td>25–52</td>
<td>2.13 (n=64)</td>
<td>0.43</td>
<td>2.44 (n=73)</td>
<td>0.88 (0.63, 1.23)</td>
<td>12% (–23%, 37%)</td>
</tr>
</tbody>
</table>

- weeks I-24, the incidence was higher in the placebo group
- ...In weeks 25-52: similar...

“At the beginning of the trial, there was evidence for Pyr-dapsone-resistant falciparum malaria at Magoda. Three of 17 cases with pure falciparum infections treated with Pyr-dapsone during the first 4 weeks of the trial failed to clear asexual parasitaemia by days 13 or 14 after treatment.”

Maybe drug resistance was selected for..?
Clyde used pyrimethamine prophylaxis in the 1950ies..
MDA selection of resistance

Prev. of *Pfdhps* 540E (and 5MT) in 1997 at 64/76 = 84.2%
Before SP was implemented in Tanzania..

*Alifrangis AJTMH 69(6), 2003, pp. 601–606*