SCHOOL OF HEALTH TECHNOLOGY
LECTURE SERIES

ANTIMALARIAL DRUG RESISTANCE: MOLECULAR CONSIDERATIONS AND IMPLICATIONS FOR EVIDENCE BASED RESEARCH IN NIGERIA

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Antimalarial drug resistance: molecular considerations and implications for evidence based research in Nigeria by Chukwuocha, U. M. is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License.
**THE CONCEPT AND THE DISEASE**

- A mosquito-borne infectious disease of humans and other animals caused by parasitic protozoans of the genus *Plasmodium* of which *P. falciparum* is most common here.

- Transmitted via a bite from an infected female Anopheles mosquito (*Anopheles gambiae sensu stricto*), which introduces the organisms from its saliva into a person's circulatory system.

- Malaria causes symptoms that typically include fever and headache, which in severe cases can progress to coma or death.

- Complications include cerebral, renal involvement, anemia, hypoglycemia etc.
Other non Classical Symptoms also exist.

The disease is widespread in tropical and subtropical regions in a broad band around the equator, including much of Sub-Saharan Africa, Asia, and the Americas. Rainfall, warm temperatures, and stagnant waters provide habitats ideal for mosquito larvae in these areas.

The World Health Organization has estimated that in 2010, there were 219 million documented cases of malaria. That year, the disease killed between 660,000 and 1.2 million people (WHO, 2010).

The actual number of deaths is not known with certainty, as accurate data is unavailable in many rural areas, and many cases are undocumented.

Malaria is commonly associated with poverty and may also be a major hindrance to economic development.
Clinical manifestations

- Microscopic examination of blood using blood films,
- Antigen-based rapid diagnostic tests.
- Polymerase chain reaction to detect the parasite's DNA have also been developed.
TREATMENT

- Antimalarial medications
- Quinine
- Chloroquine
- Antifolates (Sulphadoxine Pyrimethamine)
- Artemisinin-Based Combination Therapies
- Herbal Therapies

✓ Home Management of Malaria

Soul Beat Africa Malaria Network, 2010
Makoah, and Gabriele (2013)
PREVENTION AND CONTROL

- Vector Control/Environmental Management
- Insecticide Treated Beds/Materials/Curtains
- Indoor Residual Spraying
- Intermittent Preventive Treatment (Medication)
- Vaccine
Recurrent Malaria

- Recrudescence
- Relapse
- Re-infection

Genetic Resistance

- Several genetic factors provide some resistance to it including sickle cell trait, thalassaemia traits, glucose-6-phosphate dehydrogenase deficiency, and the absence of Duffy antigens on red blood cells
ANTIMALARIAL DRUG RESISTANCE

- Anti-malarial drug resistance has been defined as: "the ability of a parasite 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."
  
  White, 2004

- In order for a case to be defined as resistant, the patient under question must have received a known and observed anti-malarial therapy.

- There is a difference between treatment failure and drug resistance.

- It is generally accepted to be initiated primarily through a spontaneous mutation that provides some evolutionary benefit, thus giving an anti-malarial used a reduced level of sensitivity.

- This can be caused by a single point mutation or multiple mutations.
Recent reports from Guinea Bissau (Ursing et al, 2011) and Malawi (Laufer, 2006; Laufer et al, 2012) however indicate the gradual disappearance of resistance to chloroquine based on molecular studies.

Evidence of development of resistance against ACTs in parts of Asia has also been documented (Breman, 2012; Amatarunga et al, 2012).
FACTORS CONTRIBUTING TO THE SPREAD OF RESISTANCE

**Human Behaviour - Drug Pressure due to**
- Complacency
- Indiscriminate drug usage
- Adulterated Drugs

**Biological Influences**
- Pharmacokinetics of drugs and reaction with parasite
- Decreased Immunity
- Malnutrition
- Cross reactivity with drugs for other ailments
TOOLS FOR MONITORING RESISTANCE

The increasing spread of antimalarial drug resistance has emphasized the need for systematic monitoring to suggest where malaria treatment policies should be revised to secure rational use and effective case management.

The available monitoring procedures include

- **Therapeutic efficacy test** (also known as the *in vivo* test), which involves the repeated assessment of clinical and parasitological outcomes of treatment during a fixed period of follow-up to detect any reappearance of symptoms and signs of clinical malaria and/or parasites in the blood.
- *in vitro* studies of parasite susceptibility to drugs in culture
- Case detection
- Molecular methods of gene mutations or gene amplifications associated with parasite resistance
**DRUG RESISTANCE GENES AND RESISTANCE MECHANISMS**

**PfCRT - P. falciparum chloroquine resistance transporter gene**

- Several biochemical studies of the parasitic Digestive Vacuole, comparing parasites expressing a mutant or a wild type pfcrト allele, demonstrated that chloroquine accumulates within the DV and that parasites with mutant PfCRT accumulate less chloroquine than parasites expressing wild type PfCRT.

- The most plausible explanation for this difference in accumulation is that chloroquine resistant parasites can export chloroquine via active transport. PfCRT haplotypes also influence susceptibility to other antimalarial drugs, including amodiaquine, quinine and lumefantrine.

Sanchez et al, 2007; Sanchez, Stein and Lanzer, 2007; Sanchez et al, 2005; Yayon et al, 1984
Dehydrofolate Reductase (PfDHFR) and Dehydropteroate Synthase (PfDHPS) Mutations

- The folate pathway provides the parasite with cofactors that are essential for the production of pyrimidines for DNA replication and the metabolism of several amino acids. Two enzymes, dihydropteroate synthase (PfDHPS, PF08_0095) and dihydrofolate reductase activity are targeted by antifolate drugs.

- Resistance to this safe and affordable combination therapy sulfadoxine–pyrimethamine (SP, also known as Fansidar) has emerged in the late 1980s and is now widespread with point mutations in both pf dhfr and pf dhps implicated in resistance.
**P. falciparum multidrug resistance transporter 1 (pfmdr1)**

- It has been demonstrated that PfMDR1 resides like PfCRT, within the membrane of the DV.

- Mutations in MDR transporters lead to a decreased intracellular drug accumulation, increased drug efflux, and cross resistance to structurally unrelated drugs.

- From analysis of field isolates, five amino acid positions (86, 184, 1034, 1042 and 1246) have been reported to influence susceptibilities to lumefantrine, artemisinin, quinine, mefloquine, halofantrine and chloroquine.

Sisowath et al, 2005; Sidhu et al, 2005; Pickard et al, 2003; Reed et al, 2000

Plowe, 2007
Other Antimalarial Drug Resistance Polymorphisms

- Candidate Genes identified during search for loci associated with established resistance markers but their activities are not yet clear;
- The *P. falciparum* Na+/H+ exchanger (*pfnhe*) associated with decreased quinine susceptibility.
  
  *Raj et al, 2009*

- The multidrug resistance-associated protein (*PfMRP*) associated with reduced susceptibility to chloroquine and quinine.
  
  *Mu et al, 2003*

- Cytochrome bc1 complex mutations associated with atovaquone resistance
  
  *Barton et al, 2010*
Study Site and Participants

- The study was conducted between February, 2011 and March, 2012 in Owerri, Imo state located in South-eastern Nigeria. Isolates of *P. falciparum* was obtained from 200 children under five years of age with microscopically confirmed, uncomplicated malaria. Attending paediatric clinics in Federal Medical Centre; Owerri after informed consent was obtained from parents or caretakers. Finger-prick blood samples were collected on filter paper (Whatman 3MM) for molecular typing.
DNA was extracted from blood spots on filter paper (QIAmp; Qiagen, Germany) according to manufacturer’s instructions. Pfmdr1 alleles at codons 86, 186, 1034, 1042, and 1246 were typed by established real-time PCR assays and melting curve analysis on a Roche Light Cycler 480.

Pfcrt K76T as well as pfldhr/pfdhps were identified by nested PCR assays and subsequent Restriction Fragment Length Polymorphisms. Fragments were separated by gel-electrophoresis.

Dippmann et al, 2008

Rubio et al, 2002
### RESULTS

**Table 1: Demographic and Clinical profile of Study children**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>200</td>
</tr>
<tr>
<td>Age (months; Median, range)</td>
<td>102 (4 - 300)</td>
</tr>
<tr>
<td>Sex; M:F</td>
<td>104:96</td>
</tr>
<tr>
<td>Axillary temperature (°C, median, range)</td>
<td>36.2 (35.8 – 40.2)</td>
</tr>
<tr>
<td>Fever (%)</td>
<td>24.7</td>
</tr>
<tr>
<td>History of fever in last 2 days (%)</td>
<td>43.1</td>
</tr>
<tr>
<td>Plasmodium falciparum infection (%)</td>
<td></td>
</tr>
<tr>
<td>by Microscopy</td>
<td>68</td>
</tr>
<tr>
<td>by PCR</td>
<td>100</td>
</tr>
<tr>
<td>Mean parasite density (parasite/µl)</td>
<td>3508</td>
</tr>
<tr>
<td>Current malaria episode (%)</td>
<td>42.5</td>
</tr>
<tr>
<td>Reported treatment with antimalaria in last two weeks (%)</td>
<td>10.3</td>
</tr>
<tr>
<td>Child reportedly used bed nets last night (%)</td>
<td>6</td>
</tr>
</tbody>
</table>
Figure 1: Prevalence of Pfmdr1, Pfcrtr, Pfhdhr, and Pfhdhs polymorphisms in study children.
### Table 2: Prevalence of mixed polymorphisms in Pfmdr1, Pfdhfr, and Pfdhps

<table>
<thead>
<tr>
<th>Gene</th>
<th>Genotype</th>
<th>Prevalence N=200(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pfmdr1</td>
<td>Triple mutation (86 – 184 – 1246)</td>
<td>54(27)</td>
</tr>
<tr>
<td>Pfdhfr</td>
<td>Double mutation (108-51)</td>
<td>68(34)</td>
</tr>
<tr>
<td></td>
<td>Triple mutation (108-51-59)</td>
<td>128(64)</td>
</tr>
<tr>
<td>Pfdhps</td>
<td>Double mutation (437-540)</td>
<td>62(31)</td>
</tr>
<tr>
<td></td>
<td>Triple mutation (437-540-581)</td>
<td>122(61)</td>
</tr>
<tr>
<td>Pfdhfr/Pfdhps</td>
<td>dhfr double + dhps double or triple</td>
<td>40(20)</td>
</tr>
<tr>
<td></td>
<td>Quintuple(dhfr triple + dhps double)</td>
<td>62(31)</td>
</tr>
<tr>
<td></td>
<td>Sextuple(dhfr triple + dhps triple)</td>
<td>64(32)</td>
</tr>
</tbody>
</table>
IMPLICATIONS OF RESULTS

- The presence of molecular markers associated with antimalarial resistance among under-five children in South Eastern Nigeria was established.
- The continuous use of SP may explain the high prevalence of pfdhfr and pfdhps genes observed in the study.
- Lower frequencies of pfCRT observed may suggest the need to reexplore chloroquine as first-line treatment.
- The pattern of pfmdr1 genes is suggestive of emerging resistance for ACTs and this requires urgent attention.
Evidence-based research in Nigeria?

- Between 2001 and 2006, 68 countries (nearly 40 in Africa) have changed their policies.

WHO, 2007

- Although it might be clear in those circumstances that a first-line treatment is ineffective, the choice of a replacement drug proves difficult and slow.

- The need for high-quality monitoring of antimalarial drug resistance has increased in recent years.

- Sustained funding and encouragement of high-level research for routine monitoring activities is a major issue.

- Limited numbers of qualified personnel.
CONCLUSION AND RECOMMENDATIONS

- Our report as corroborated by others previously cited underscores the need for continuous surveillance of molecular drug resistance markers in area to inform better planning and effective/timely implementation of drug policies based on evidence.

- This will help in the timely detection of resistance for any particular drug in use at any point in time so that changes in drug policy can be made in time too.

- There is need to routinely evaluate and update our malaria treatment policies.

- It is also very important that an effective mechanism be put in place to adequately communicate especially the grass roots whenever there is change in treatment policy as well as to see to their proper implementation.

- These can only be achieved by establishing specialized laboratories in Nigeria where advanced research can be carried out and where we can also develop our own protocols based on our peculiarities.

- Adequate funding and capacity building is key.
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