Utilising sentinel travellers to detect drug resistant malaria.

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Summary

Molecular surveillance is an important tool in forestalling drug resistance. In the case of antimalarial resistance, these investigations exclude the poorest regions. My research utilises traveller's malaria (entering Australia with immigrants and refugees) to include these impoverished endemic regions in surveillance and data gathering efforts. In light of the advent of resistance to the global frontline antimalarial (artemisinin) this resistance screening will have the specific benefits of (1) pre-empting local treatment failure, (2) informing drug policies in poorer regions (most effected by malaria), as well as the broader benefit of (3) gathering data to inform malaria control and elimination.

Research Work

The project completed this year aimed to characterise two genes associated with antimalarial resistance within *P. falciparum* positive blood samples (n=153) archived with the NSW parasitology reference lab. This cohort represented the imported cases of *P. falciparum* malaria in NSW from 2010 to 2016, largely originating in sub-Saharan Africa. The project amplified the kelch propeller domain (containing mutation sites shown to confer resistance to artemisinins [1, 2]) as well as the area around codon 76 of the chloroquine resistance transporter gene [3]. These gene segments were then analyzed for resistance-associated mutations by sequencing, and allele-specific restriction digestion respectively.

The amplification and variant analysis protocols were developed and optimized to determine specificity and sensitivity detecting variants. This was conducted to appraise protocol use in a clinical setting. Clinical application was validated for the didactic purposes of revealing risk of treatment failure locally, as well as to establish confidence in the accuracy of reported findings on regional resistance trends.

The kelch surveillance protocol revealed seven propeller domain mutations. These included the C580**Y** coding mutation most strongly associated with artemisinin resistance in South East Asia [1], found in a sample originating in Papua New Guinea (where there is currently no reported resistance).

The chloroquine resistance transporter investigation yielded regional chloroquine resistance genotype proportions for the cohort's African malaria origins. A statistically significant difference was found when comparing the observed proportions to the expected proportions (from regional data available) for Nigeria, Ghana, Uganda and Sudan. As the available data is limited (often not recent and gathered from a single urban site) disagreement with the mutation proportions reported in the literature foregrounds the insufficiency of current reported data.

Future research aims to include additional molecular markers of resistance to create a screening panel, and to develop a multiplex PCR protocol to conduct this surveillance in the fastest and most cost effective manner. Additionally, I plan to culture clinical and laboratory strains of *P. falciparum* with the aim of linking *in vitro* drug resistance results to panel outcomes, validating the use of the protocol for pre-empting treatment failure.

Target Group

While it is estimated half of the world is at risk of acquiring a malaria parasite, 88% of clinical disease cases and 90% of deaths occur in Africa [4]. As with most parasites, the disease disproportionally affects the regions and people of lower socioeconomic status.

The bias in disease outcomes more significantly impacting poorer individuals is emphasised by the parasite's relationship with the immune system. Most clinical disease follows *Plasmodium* infection of an individual without immune-competency – in most case pregnant women, children under five, and those with co-morbidities – groups at an economic disadvantage in developing countries [5].

Drug resistance emphasises the skew in negative disease outcomes for groups of low socioeconomic status further. In poorer regions, the recommended combination drug therapies are often unavailable or unaffordable. This leads to the use of monotherapies of past-generation antimalarials such as chloroquine, creating a selective pressure that propagates drug resistance. Drug resistance in turn hinders elimination and control efforts preventing a reduction in endemnicity for the region [6].

Due to the effect *P. falciparum* malaria has on fertility, and the disability-adjusted years of mean lifetime labour within malarious communities [7], population and economic growth are inexorably tied to (and limited by) malaria. External intervention of this cycle is required to cease this dual effect of disease and poverty.

How the research could benefit the target group

The lack of current data on regional drug susceptibility for the poorest malarious regions represents a significant hurdle to the goal of controlling and eliminating malaria. For several impoverished areas of South East Asia, Oceania, and sub-Saharan Africa, accurate molecular data is needed to best utilise the limited anti-malaria resources available. Drug resistance patterns would highlight loci where more expensive active investigation is needed, and contribute to global data gathering efforts, including currently excluded areas.

African endemic regions in particular require the application of molecular surveillance, as immunity is likely to mask initial cases of drug-resistant parasites emerging. Molecular surveillance would identify resistance genotypes early, providing the opportunity to eliminate parasites before they disseminate resistance. Genetic studies are also the singular alternative to impractical (to the point of infeasible) on-going pan-African pharmacokinetic studies. As such, it is a priority to find a way to move forward with molecular analysis, despite the difficulties inherent conducting research in many sub-Saharan African nations.

The research already conducted has included several nations (including Nigeria, South Sudan and Sierra Leone) which were previously unsurveyed in artemisinin resistance data gathering efforts. With no other frontline therapy available as an alternative to Artemisinin Combination Therapy, forestalling the spread of resistance is imperative to upholding the progress in malaria control made over recent decades. The most imperilled regions are unable to conduct the necessary genetic investigations. This makes traveller's malaria a valuable resource, and places a social onus on countries with access to this resource (and other financial and technical resources) to collaborate internationally and conduct molecular investigation.

The gap in molecular data additionally hinders our understanding of multi-drug resistant parasites, so beyond forestalling artemisinin resistance spread, research that facilitates a more sophisticated understanding of multi-drug resistant malaria would contribute to the efforts of reducing the malaria burden, which currently so profoundly affects the groups at risk.

References

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