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Perspective

Development of Anti-Parasitic Drug Testing

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DESCRIPTION

Every year, millions of individuals are infected with Plasmodium falciparum malaria, which also claims thousands of lives. Since practically every anti-malarial treatment used to date has selected for resistant malaria parasites, the burden of this disease is further exacerbated by drug resistance. In order to quickly identify and stop the spread of resistant parasites, especially on the African continent, it is crucial to closely monitor parasite drug responses and optimise control strategies. This is especially true when it comes to reports of parasites that are less susceptible to Artemisinin Combination Therapy (ACT).

Monitoring parasite drug responses to malaria medications entails either directly assessing the frequency of mutations linked to resistance within a population of parasites or indirectly measuring both. *Ex vivo* drug resistance assays, which do not require prior culture adaption, assess treatment response in parasites directly removed from infected patients. These assays make it possible to evaluate each component of combination therapy separately against parasites and potentially identify therapeutic efficacy declines before resistance manifests clinically and spreads widely. To assess parasite drug resistance in both lab and field settings, numerous assays have been developed. Additionally, it has been demonstrated that mutations in a variety of parasite genomic loci, including pfcrt and pfmdr1, among others, lead to anti-malarial treatment resistance. The therapeutic life of present and future treatments can be increased by regularly tracking the prevalence of these mutations over a period of years. This can also indicate changes in allele selection within a population over time.

The goal of this study was to determine whether the anti-malarial medication resistance of the malaria parasites present in This, Senegal, was increasing or decreasing over time, and whether variations in parasite drug responsiveness could be accounted for by known mutations linked to drug resistance.

Sulphadoxine, pyrimethamine, and amodiaquine replaced the monotherapy of chloroquine in Senegal's drug use. ACT (predominantly artesunate-amodiaquine in Thiès) replaced ACT once more. Drug sensitivity to parasites has already been tested in-person in Senegal, and the frequency of mutations linked to resistance has also been monitored. In order to understand how parasites in Senegal may be changing in response to medicine treatment, the goal of this study was to quantify both parasite drug sensitivity and resistance mutation prevalence over time.

Based on the culture techniques of Trager and Jensen, Rieckmann et al. developed the *in vitro* microtest. For each experiment, microtiter plates are precoated with two drug-free control wells and various doses of chloroquine. 50 ml of buffered RPMI 1640 medium and 5 ml of whole capillary blood were added to each well before being incubated at 38°C-39°C for 24-30 hours in a candle jar. Following incubation, thick blood films are made from each well, and 500 leukocytes are used as a reference point to count the amount of schizonts. The proportion of schizonts at each drug concentration compared to the number of schizonts in drug-free controls, which represents the drug's reaction in terms of the number of schizonts. Using normal RPMI 1640 with 1 mg/l folic acid and 1 mg/l para-amino benzoic acid, this approach is also used to assess the *in vitro* response of the malarial parasite to pyrimethamine and sulfadoxine-pyrimethamine in combination. The fact that this method just needs a little amount of capillary blood and involves straightforward *in vitro* processes offers significant benefits.

Microscopists dislike this tedious process since it takes so long to complete. The schizont stage is not reached by parasites that develop from the ring to late trophozoite stage in less than 24 hours. These 24 hours of incubation assays may have difficulty measuring medications like sulfadoxine and pyrimethamine, which typically act during later stages of parasite development. Based on the measurement of the growth in parasitemia throughout a culture, the incubation period for numerous antimalarial drug susceptibility assays increased to 48-96 hours with the invention of continuous in vitro cultivation method of

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P. falciparum. However, it still takes a lot of time and effort to interpret test results. The development and maturation of parasites *in vitro* culture media with various known concentrations of antimalarial medicines is the primary foundation for all susceptibility assay methods for antimalarial medications. Each approach has unique costs, feasibility, and sensitivity. For a while, the radioisotope assay method was regarded as the gold standard, but its widespread use was constrained by the risks involved with radioactive waste disposal. Fluorometric assay and flowcytometry are two methods for assaying with DNA-specific fluorochromes that are quick, accurate, highly sensitive, highly DNA-specific, objective, and automated. However, these methods require expensive equipment and have a high background noise level when leukocytes are present. Therefore, additional research using SYBR Green I fluoroassays for field isolates is required. These drawbacks may be overcome by two newly created ELISA-based colorimetric assays (DELI and HRP II assays), which are now the preferred techniques for susceptibility assay. These are less complicated, easier to use in the field, and require less specialist equipment than other approaches. They are also more sensitive than other methods.