Drug Resistance Profiling of Malaria Isolates in the BEI Resources Repository

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Malaria control and elimination efforts are hampered by widespread resistance to frontline antimalarial drugs. Resistance has developed to all antimalarial drugs in clinical use. BEI Resources, a NIAID-funded program managed by ATCC, serves researchers worldwide through the provision of drug-resistant reference strains, which are critical for the development of diagnostics, vaccines and therapeutics, and are available to qualified registered investigators and institutions. Bioresources within BEI include well-characterized malaria isolates as part of the Malaria Research and Reference Reagent Resource Center (MR4). Concerted efforts are made to acquire, produce, authenticate (characterize), preserve, and distribute drug-resistant strains of topical interest to the malaria research and control community. As part of our efforts to provide well-characterized drug-resistant reference isolates, we have been measuring in vitro antimalarial susceptibility of malaria isolates acquired from different malaria-endemic countries since 2012. SYBR® Green I-based IC50 assays were used to determine the susceptibility of >100 catalog isolates against a standard panel of drugs, including chloroquine (CQ), artemisinin (ART), quinine (QN), pyrimethamine (PYR), sulfadoxine (SDX), and cycloguanil (CYC). Additional antimalarial susceptibility testing was performed against a secondary panel of drugs, including halofantrine (HF) and lumefantrine (LM). Because standard IC50 assays poorly capture in vitro parasite susceptibility to artemisinin drugs and piperaquine, ring-stage survival assays and piperaquine-survival assays were also performed on a subset of isolates (n = 22), which were primarily deposited into BEI Resources as showing sensitivity or resistance to these drugs. Our results reveal a range of drug susceptibility profiles for the different isolates and provide valuable information to assist current and prospective users of the BEI Resources repository in making data-driven requests of isolates to meet their research needs.

BACKGROUND

- Malaria control and elimination efforts are hampered by widespread resistance to front-line antimalarial drugs.
- Funded by the National Institute of Allergy and Infectious Diseases (NIAID), BEI Resources provides registered researchers and institutions worldwide with a centralized repository for the acquisition, production, characterization, preservation, storage and distribution of well-characterized drug-resistant malaria strains. The Malaria Research and Reference Reagent Resource Center (MR4) integrated with BEI Resources in 2010.
- The present study measured *in vitro* antimalarial susceptibility profiles of >100 strains in the BEI Resources Catalog with the goal of helping registered and prospective users make judicious data-driven requests of isolates.

MATERIALS & METHODS

BEI Resources *Plasmodium falciparum* strains used

- 109 *P. falciparum* isolates in the BEI Resources Catalog were assayed for *in vitro* drug sensitivity.
- These include the widely used 3D7 (MRA-102) and Dd2 (MRA-150) parasites.
- Parasites were grown in leukocyte-depleted human type O+ erythrocytes.

Standard *in vitro* antimalarial susceptibility testing

- *In vitro* susceptibilities of isolates were measured against standard antimalarial compounds including chloroquine (CQ), quinine (QN), artemisinin (ART), pyrimethamine (PYR), sulfadoxine (SDX) and cycloguanil (CYC) using the standardized SYBR Green Antimalarial Assay[1,2]. A subset of isolates (n = 26) were also tested against a secondary drug panel (lumefantrine and halofantrine).
- Antimalarial test compounds were obtained from the WorldWide Antimalarial Resistance Network (WWARN), Sigma-Aldrich, Toronto Research Chemicals (TRC) and MP Biomedicals.
- Drug assays were run in triplicate at 0.5% parasitemia and 1.5% hematocrit in 96-well plates. Each assay run included laboratory control parasite lines, 3D7 (MRA-102) and Dd2 (MRA-150).
- Dose-response curves and half-maximal inhibitory concentrations (IC_{50}s) for each drug were determined from the parasite growth inhibition data using GraphPad Prism v8.0.

***In vitro* Antimalarial Susceptibility Testing using Parasite Survival Assays**

- Because IC_{50} Assays fail to accurately capture parasite susceptibility to artemisinins (ARTs) and piperaquine (PPQ), susceptibility to these drugs was measured using Parasite Survival Assays.[3,4]
- Parasites were exposed to a pharmacologically-relevant drug dose (700nM DHA for 6 h and 200nM PPQ for 48 h) in 48-well plates under standard culture conditions. Drug was then removed, and parasites were allowed to recover and proliferate in normal growth media.
- % Parasite survival was determined by microscopic examination and counting of viable parasites in drug-treated versus untreated wells. Parasites showing a survival rate of ≥10% were deemed drug-resistant.

TECHNICAL APPROACH SUMMARY

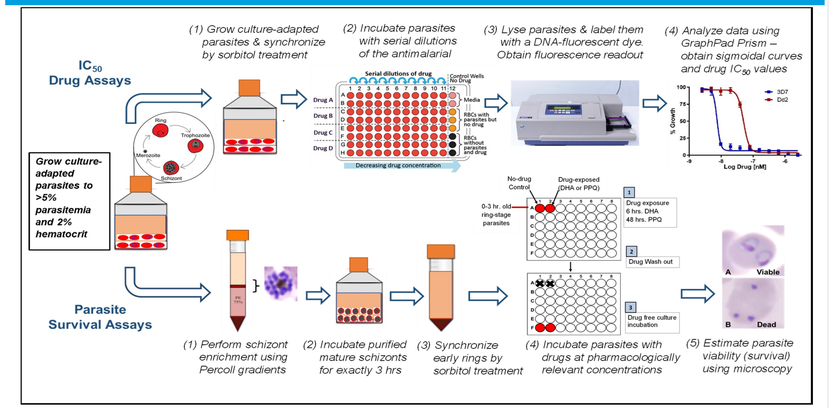

Figure 1. Schematic representation of antimalarial susceptibility testing for malaria isolates using standard IC50 Drug Assays (Top panel; steps 1 – 4) and Parasite Survival Assays (Bottom panel; steps 1 – 5). Drug concentrations were converted to their natural logarithm to plot the sigmoidal curves and estimate IC50 of each compound

RESULTS

Malaria isolates in the BEI Resources Catalog exhibit a range of antimalarial IC_{50} values

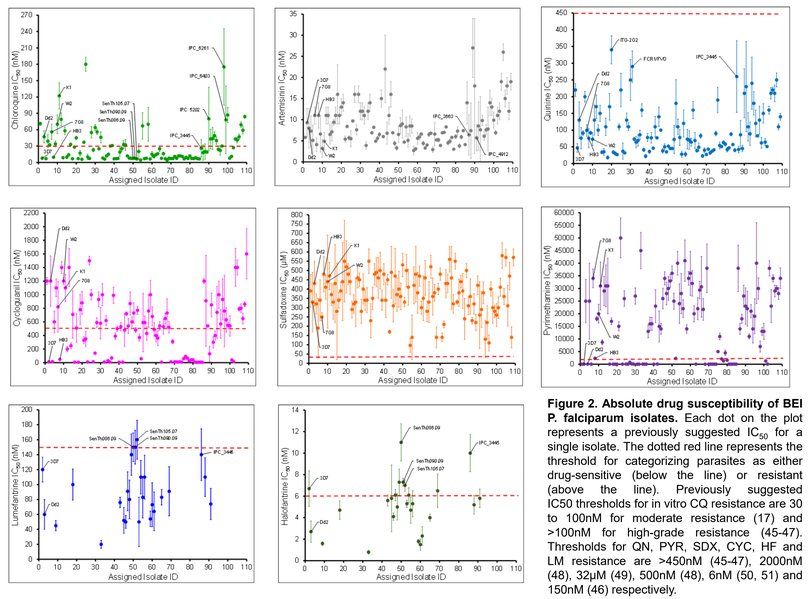

Figure 2. Absolute drug susceptibility of BEI *P. falciparum* isolates. Each dot on the plot represents a previously suggested IC_{50} for a single isolate. The dotted red line represents the threshold for categorizing parasites as either drug-sensitive (below the line) or resistant (above the line). Previously suggested IC50 thresholds for in vitro CQ resistance are 30 to 100nM for moderate resistance (17) and >100nM for high-grade resistance (45-47). Thresholds for QN, PYR, SDX, CYC, HF and LM resistance are >450nM (45-47), 2000nM (48), 32µM (49), 500nM (48), 6nM (50, 51) and 150nM (46) respectively.

RESULTS

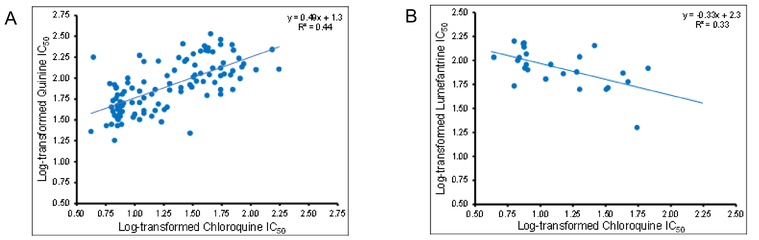

Figure 3. Correlation between in vitro activities of antimalarial drugs. Scatter plots of IC_{50} data recapitulate previously defined activity relationships between drugs. This includes the positive correlation between the potency of chloroquine (CQ) and quinine (Panel A), and the inverse relationship between the in vitro activities of CQ and lumefantrine (Panel B).

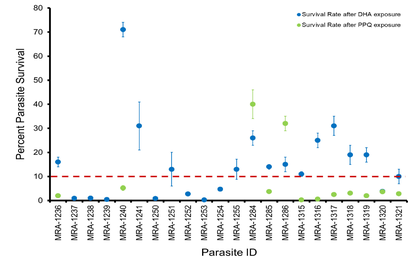

Figure 4. Parasite Survival Rates after exposure to pharmacologically relevant DHA or PPQ concentrations. Blue and green dots show data for DHA and PPQ respectively. The dotted red line is a previously defined threshold for categorizing parasites as either drug-susceptible (below the line) or drug-resistant (above the line). Both susceptible and resistant isolates are adequately represented in the BEI Resources MR4 repository

SUMMARY

- Over a hundred isolates with unique *in vitro* antimalarial susceptibility profiles are available to the malaria research community through NIAID's BEI Resources Program (https://www.beiresources.org)
- Standard IC_{50} assays reveal significant variation in drug susceptibility among BEI Resources *P. falciparum* isolates (Fig. 2). This extensive variation in drug response was observed across all the drugs tested except quinine, which was potent against all the isolates, and sulfadoxine, which was ineffective against all the isolates analyzed (Fig. 2).
- The drug susceptibility status of each isolate matched the drug response phenotype indicated by the isolate's depositor.
- We observed that absolute IC_{50} values vary significantly even within the two defined categories of susceptibility and resistance, suggesting a multigenic inheritance of drug resistance.
- Parasite survival assays are more informative than traditional IC_{50} assays at assessing ART and PPQ susceptibility.

References

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