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The Impact of Heterogeneous Transmission on the Establishment and Spread of Antimalarial Drug Resistance

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Abstract

Despite the important insights gained by extending the classical models of malaria, other factors, such as immunity, heterogeneous biting, and differential patterns of drug use have not been fully explored due to the complexity of modeling multiple simultaneous malaria infections competing within a host. Understanding these factors is important for understanding how to control the spread of drug resistance to artemisinin which is just emerging in Southeast Asia. The emergence of resistance plays out at the population level, but is the result of competition within individuals for transmission events. Most studies of drug resistance evolution have focused on transmission between hosts and ignored the role of within-host competition due to the inherent complexity of modeling at multiple scales. To embed within-host competition in the model, we used an agent-based framework that was developed to understand how deviations from the classical assumptions of the Ross-MacDonald type models, which have been well-described and analyzed, impact the dynamics of disease. While structured to be a stochastic analogue to classical Ross-Macdonald type models, the model is nonetheless based on individuals, and thus aspects of within-host competition can be explored. We use this framework to explore the role of heterogeneous biting and transmission on the establishment and spread of resistance in a population. We find that heterogeneous transmission slows the establishment of resistance in a population, but once resistance is established, it speeds the spread of resistance through the population. These results are due to the skewed distribution of biting which makes onward transmission a low probability and suggests that targeting the “core” group of individuals that provide the vast majority of bites could significantly slow the spread of resistance.

Keywords

Malaria; Agent-based models; Drug Resistance; Epidemiology; *Plasmodium falciparum*

Introduction

Effective antimalarial drugs have saved millions of lives since their use became widespread in the 1950s. Unfortunately the emergence of resistance to the first-line drugs chloroquine (Payne, 1987) and then sulphadoxine-pyrimethamine (Nair et al., 2003; Roper et al., 2003)

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significantly undermined the ability to control the disease (Wongsrichanalai et al., 2002) and increased morbidity and mortality (Snow et al., 2001; Trape et al., 1998). In recent years, global efforts have been directed at maintaining the effectiveness of the artemisinin drugs, which in combination with another drug are the current recommended first-line therapy for uncomplicated malaria. While strategies, such as combination therapy, can serve to delay emergence (Smith et al., 2010a; White, 1999a; White, 1999b), the evolution of resistance is likely inevitable. In fact, an artemisinin delayed clearance phenotype has already been reported (Dondorp et al., 2010), suggesting that resistance to artemisinin drugs may already be emerging. As such, a better understanding of how resistance becomes established and spreads through a population is necessary for devising future policies and interventions to deal with the inevitable.

Since the early 1900s mathematical models of malaria have been used to understand the dynamics of the disease and guide policy (Smith et al., 2012). In recent years a number of different models have elucidated important aspects of the evolution of resistance, particularly regarding the role of transmission intensity, superinfection (i.e. multiple simultaneous malaria infections (Macdonald, 1950)), and clinical immunity (Artzy-Randrup et al., 2010; Chiyaka et al., 2009; Hastings, 1997; Hastings, 2006; Hastings and D'Alessandro, 2000; Klein et al., 2008; Pongtavornpinyo et al., 2008). However, most of these theoretical studies of drug resistance evolution have focused on transmission between hosts and ignored the role of within-host competition (Klein, 2013; Reiner et al., 2013), which, while simplifying the dynamics, makes it difficult to understand how the dynamics are impacted by competition within the host. In an agent-based framework, it is possible to embed within-host competition; however this creates a degree of complexity that makes it difficult to analyze the model. To bridge this difference, we have developed an individual-based model that is a direct analogue of a mathematical model of mosquito-borne pathogen transmission based on the assumptions of the Ross-MacDonald model. The model builds on prior epidemiological models of vector-borne transmission (Bailey, 1982; Dietz et al., 1974; Macdonald, 1950), and takes a stepwise approach to incorporating both competition and drug resistance. This makes the analysis tractable and allows for a careful examination of how deviations from the orthodoxy of the Ross-Macdonald framework, in particular the role of heterogeneous biting, impacts the dynamics of infection and the establishment and spread of drug resistance.

Methods

Malaria is a vector-borne disease transmitted by the bite of an anopheles mosquito. Individuals living in malarious regions of the world are bitten regularly by infected mosquitoes. This continuous re-exposure results in simultaneous infection, or superinfection (Macdonald, 1950), with multiple parasites. The number of genetically distinct parasite genotypes, called the multiplicity of infection (MOI), generally increases on average in the population with transmission (Bendixen et al., 2001; Sama et al., 2006), but varies based on mosquito biting patterns (Smith et al., 2005) and the age of the individual (Bendixen et al., 2001; Sama et al., 2006), which likely has to do with the level of acquired immunity (Klein, 2013). We base our assumptions of how parasites compete within the host on a Markov-Chain model for superinfection and clearance developed by Bailey (Bailey, 1982) based on work by MacDonald, Irwin, Fine and Walton (Dietz et al., 1974; Fine, 1975; Macdonald, 1950; Walton, 1947). We first describe a class-based deterministic model that will serve as the basis for comparison to an agent-based model.

Mosquito Dynamics

To ensure that our formulation will be analogous to an individual based version, we explicitly model the mosquito population's contribution to transmission following classical

malaria notation (Smith and McKenzie, 2004). Susceptible mosquitoes (M_S) emerge at a constant rate per human (m) and are assumed to die at a constant rate (g), which generates an equilibrium mosquito density per human (m/g). The rate that mosquitoes become latently infected (M_L) is dependent on the density of mosquitoes, the biting rate (a) and the efficiency of transmission from human to mosquito (c). Mosquitoes that become infected must then survive sporogony (n) before becoming infectious (M_I). The dynamics of the mosquito population are described by the following set of ordinary differential equations:

$$\begin{aligned}\dot{M}_S &= mN_H - gM_S - \frac{acI_H}{N_H} M_S \\ \dot{M}_L &= \frac{acI_H}{N_H} M_S - (g+n)M_L \\ \dot{M}_I &= nM_L - gM_I\end{aligned}\quad (1)$$

where N_H is the total number of humans and I_H is the total number of infected humans.

Human Dynamics

The human dynamics are formulated so that $\sum_i I_i = 1$ and $\sum_i \dot{I}_i = 0$, where I_0 are susceptible individuals. Thus, the values of the state variables describe the distribution of susceptible and infected individuals in the population over time. Humans are infected by infectious mosquitoes at a rate dependent upon the mosquito density, the biting rate, and the infectivity rate (b), or the fraction of bites on humans that produce a patent infection. The equation describing the dynamics of the population of susceptible humans is,

$$\dot{S} = \dot{I}_0 = B - \left(\frac{abM_I}{N_H} + \mu \right) I_0 + \gamma I_1 + \psi \rho \sum_{j=1}^n I_j \quad (2)$$

and the equations describing infected humans is,

$$\dot{I}_j = \frac{abM_I}{N_H} I_{j-1} - \left(\frac{abM_I}{N_H} + \mu + \gamma j + \psi \rho + \delta \right) I_j + \gamma(j+1) I_{j+1}, \quad j \geq 1 \quad (3)$$

where μ is the background mortality rate, γ is the rate infections are cleared, and δ is the disease induced death rate. We also assume that deaths are balanced by births (B). Lastly, drug use is assumed to be associated with clinical symptoms (primarily fever), which develops at rate ψ . The rate that clinical symptoms arise is assumed to be independent of MOI. A fraction of symptomatic patients, ρ , are assumed to use drugs and successfully clear all sensitive parasites immediately upon treatment. Treatment of resistant parasites is assumed to be ineffective. For numerical simulations we assume that the max MOI is 100, thus to avoid a population leak, the equation for the maximum MOI differs slightly,

$$\dot{I}_{j_{max}} = \frac{abM_I}{N_H} I_{j_{max}-1} - (\mu + \gamma j_{max} + \psi \rho + \delta) I_{j_{max}} \quad (4)$$

though this has negligible impact because of the high assumed MOI. See Figure 1 for a schematic of the model.

Computationally Efficient Individual-Based Model

Our individual model of malaria was developed to be, in its base form, a stochastic analogue of the deterministic model described above. Thus, the model is composed of individual humans that can be infected through infectious bites from mosquitoes and individual

mosquitoes that can be infected by biting infected humans. Simulation of the model is based on Gillespie's Direct Method, an algorithm that can generate statistically rigorous solutions to systems of stochastic equations (Gillespie, 1976; Gillespie, 1977). Originally developed for simulation of chemical reactions, it has been used extensively in examining biological questions. The basic premise of the algorithm is that the transition of a homogenous mixture of species from one state to another can be described by probabilistic methods.

In the Direct Method of the algorithm, the system is started in an initial state and then the next reaction and the time to that reaction are selected by sampling from probability distributions that describe the temporal behavior of the system (Gibson and Bruck, 2000). Thus, to implement the Direct Method all that is needed to find the state of a system given its current state is to calculate what and when is the next event (Gillespie, 1977). Despite the ability of the Direct Method to calculate statistically rigorous solutions, it requires a significant amount of computational power when the system is complex or the population is large. Translation of the Gillespie Algorithm to individual agents, where the rate at which events occur are specific for each agent, creates another scaling problem as the algorithm requires every rate for every individual to be calculated after every event. Though technically possible, as the population increases linearly, the computational time increases exponentially (Allen and Dytham, 2009).

A computationally efficient alternative was developed by Allen and Dytham (2009) specifically directed at modeling biological populations. While exactly equivalent to the Direct Method, this alternative is significantly more computationally efficient. The difference lies in the fact that the computational cost per event in the alternative algorithm is independent of population size (Allen and Dytham, 2009). As with the Direct Method, time steps are sampled from an exponential distribution of probabilities that are analogous to rates. However, in this case the rates refer to the maximum rate (c_x) possible for each event (x) across all individuals. At each time step a potential event is identified by selecting an event based on its maximum rate and then determining whether this event is executed by selecting an individual to experience the event. Each individual has an individual probability of an event occurring (x_i), which is less than or equal to the maximum rate. The maximum rate is chosen as the maximum individual probability possible. The probability of the event occurring is the probability of the event being selected (c_x/c , where $c = \sum c_x$), the probability of the i th individual being selected, and the probability that the selected event is executed (x_i/c_x). Thus, at each time step there is no guarantee an event will occur.

Computationally Efficient Malaria Individual-Based Model

The human dynamics of the class-based model were converted to the computationally efficient algorithm by calculating the maximum possible probabilities that an individual dies (μ), becomes infected (abM_I/N_H), recovers from an infection (γ) (i.e. loses a parasite clone), becomes clinically ill (ψ) and treats (ρ), and dies from the disease (δ). The time until the next event is determined as

$$\tau = \frac{1}{c} \ln(1/r) \quad (5)$$

where r is a uniform random number, and c is the sum of all the maximum probabilities. The next possible event is then chosen by selecting a random event from all possible events weighted by their probabilities. To determine if the event occurs, a random individual from the population is selected. The probability of the event occurring is then the individual's probability for that event occurring to them divided by the maximum probability for that event. For example, if the selected event is recovery, but the individual is not infected (i.e.

their individual probability of recovery is 0), the event would not occur. However, if they were infected, then the probability of the event occurring would be their individual recovery probability divided by the maximum recovery probability. A random uniform number is then drawn to determine if the event occurs. If the event occurs, the population is updated.

Because the lifespan of the human population is significantly longer than the mosquito, and thus the dynamics operate on a much slower time-scale, we only update the mosquito dynamics after each time step, τ . This process takes advantage of the fact that over the interval $t + \tau$, the mosquito population size does not change significantly enough to impact the dynamics. For mosquito births, deaths, and sporogony, we use the τ -leap method (Gillespie, 2001). The τ -leap method is an extension of the direct method, which calculates the expected number of each event occurring over the interval $[t, t + \tau]$ and executes them all at one time. The number of times K_x that an event will occur over the interval $[t, t + \tau]$ given the state of the system X at time t can be approximated by a poisson random variable:

$$K_x(\tau; X, t) = P(a_x(X)\tau) \quad (6)$$

where a_x is the propensity function, or the probability of event x occurring in an infinitesimal amount of time, dt . The key requirement of the τ -leap method is to ensure that τ be of short enough duration that the change in the state of the system during the interval $[t, t + \tau]$ will cause no appreciable change in the probability distributions of each variable, which we ensure by updating the mosquito population after each time-step (whether an event occurred or not) of the human dynamics.

While we keep track of individual infected and infectious mosquitoes in order to track individual parasites, we assume that susceptible mosquitoes are all equal and thus don't keep track of individual susceptible mosquitoes. Thus, at every defined interval, we calculate the number of new mosquitoes that emerge using a poisson random variable, $P(mN_H\tau)$, and we also calculate the number of susceptible mosquitoes that died over this period, $P(gM_S\tau)$. The total number of susceptible mosquitoes is then updated. A similar process for the infected and infectious mosquitoes is used. For each interval, the number of latent mosquitoes that survive sporogony $P(nM_L\tau)$ become infectious, while those that die of the latent and infectious, $P(gM_L\tau)$ and $P(gM_I\tau)$, respectively, are eliminated from the population.

For the infection dynamics of mosquitoes, we use a similar process as for the humans, however in this case there is only one event, mosquito infection, which occurs with a probability of (acI_H/N_H) . Thus, we only need to (1) calculate the time until the next possible mosquito infection, if it is less than $t + \tau$ then; (2) select a random individual from whom a susceptible mosquito may acquire an infection and calculate the probability that the event occurred; (3) if it occurred, update the mosquito population; (4) update the time. We initiate these dynamics at time t and continue until the next event would be past $t + \tau$. The model was written in C++ and simulated using the parameter values detailed in Table 1.

Biological Cost of Drug-Resistance

The key aspect that the current formulation adds to the question of superinfection is the ability to simulate the impact of within-host competition on transmission events. While the number of genotypes that are transmitted from a human to a mosquito can be numerous, the ultimate number of genotypes transmitted by infectious bites of mosquitoes on humans are limited due to a winnowing process within the mosquito (Sinden and Billingsley, 2001). Thus, we assume that each mosquito ultimately transmits the offspring of only one of the parasites that infects a human host, regardless of the host's MOI, and the 'winning' parasite is determined at the time a mosquito becomes infected. This allows us to examine the

outcome of within-host competition between parasites with different phenotypes. The key phenotype that we are concerned with is the biological cost of drug-resistance.

The acquisition of drug tolerance by parasites is encoded by single mutations, multiple mutations, or changes to the copy number of genes relating to the drug's target or influx-efflux pumps that affect intraparasitic concentrations of the drug (White, 2004). These genetic changes, while providing a benefit in the presence of drugs, can also reduce the parasites competitiveness in the absence of drug therapy (Babiker et al., 2009; Felger and Beck, 2008). This biological cost of resistance reduces the reproductive efficiency of the parasite and slows its growth rate (Babiker et al., 2009), though this likely has differential impacts on transmission and clearance.

We assume that drug-resistant parasites have both “hard” and “soft” costs of resistance (Babiker et al., 2009). Hard selection is where the fitness cost of maintaining resistance genes is incurred irrespective of the presence or absence of drug-sensitive parasites. Thus, we assume that drug-resistant parasites have a greater probability of clearance at all times, whether they are competing with drug-sensitive parasites or not. Soft selection, on the other hand, is where fitness costs are only apparent in the presence of competition. In this case, we assume that drug-resistant parasites have a lower probability of transmission only when they are competing with drug-sensitive parasites in a host. We assume a soft fitness cost with respect to transmission because (a) the correlation between asexual parasite density and gametocyte density is limited (Taylor and Read, 1997), (b) transmission probability is both saturating and efficient at low densities (Barnes and White, 2005), and (c) there is ample evidence of within-host competition between clones for transmission events (Talisuna et al., 2003; Wargo et al., 2007), suggesting that it is likely that small differences in probability will result in differential transmission. For clearance, a hard cost was assumed because, as noted above, the acquisition of resistance mutations decreases the reproductive rate of the parasite (Babiker et al., 2009), which is likely to reduce the duration of infection for drug-resistant parasites regardless of competition with drug-sensitive clones. In the absence of a fitness cost, transmission is implemented by randomly selecting a parasite from an infected individual. With competition, the sampling weights of each parasite are weighted. Thus, for example, if there were two parasites, one resistant and one sensitive, in an individual, the probability of selecting the resistant one would be lower. For clearance, as noted above, each individual has different probabilities of an event occurring, thus, individuals with a resistant parasite have an increased probability of a recovery event occurring as compared to an individual with a sensitive parasite.

Heterogeneous Biting

Individuals and mosquitoes are distributed non-randomly across the landscape (Smith et al., 2004) which generates spatially heterogeneous biting patterns. These differences in biting patterns are further localized by movement patterns of both mosquitoes and humans (Stoddard et al., 2009). In addition, mosquitoes are differentially attracted to some individuals (Knols et al., 1995; Lindsay et al., 1993; Mer et al., 1947), and are more likely to feed on a larger individual (i.e. adult vs. child) because they have more surface area for biting (Port et al., 1980). Thus, it is likely that MOI is highly variable across a spatial landscape, even at the scale of a village, which would result in differential levels of competition depending upon the rate that a host is bitten.

Because our model tracks individuals, mosquitoes and parasites, we had the ability to explore in a tractable manner, the impact of heterogeneous biting on the evolution of resistance. To incorporate heterogeneous biting, we assumed that every individual has a different propensity for being bitten by mosquitoes. We assumed that the probability of being bitten by a mosquito was gamma distributed so that, as suggested, ~20% of the

individuals receive ~80% of the bites (Smith et al., 2005; Smith et al., 2010b; Woolhouse et al., 1997). Heterogeneous biting enters the model in two places, (1) when an individual infects mosquitos, and (2) when mosquitos infect individuals. At the outset of the model each individual is assigned different probabilities of being bitten, a_i . To maintain comparability to simulations assuming uniform biting, we used an overdispersed gamma function such that the mean of a_i equals the biting rate, a , used in the uniform biting simulations, $\Gamma(k = 1, \theta = 0.3)$. In the human dynamics, the probability that an individual becomes infected is thus now, $a_i b M_I / N_H$. Thus, when the simulation is run, and the infection event is selected, we calculate the probability of the event being executed as the probability of an individual becoming infected divided by the maximum probability of an individual becoming infected. Since the probability of an individual becoming infected is less than or equal to the maximum probability of infection, the lower a_i is relative to the maximum a_i , the lower the probability an individual will become infected (see description of event probabilities above). Similarly, in the mosquito dynamics, the probability that a mosquito becomes infected by an individual is now $a_i c I_H / N_H$, and the lower a_i is relative to the maximum a_i , the less likely that individual is to infect a mosquito.

Results

To demonstrate the ability of the model to approximate the dynamics of the deterministic superinfection model, we simulated the system 10,000 times with a population size of 10,000. To avoid the disease stochastically dying out before an epidemic begins, we started with five infected individuals (0.05% of the population). We assumed, as in the deterministic superinfection model that all parasite clones are phenotypically the same (Bailey, 1982; Macdonald, 1950), thus, the probability of any single parasite being selected when a mosquito bites a human is equal. Clearance was also similar to the deterministic superinfection model in that an individual human's probability of clearing a parasite clone on any day is related to the number of clones they harbor. Thus, if they harbor ω clones, the individual probability of clearance is equal to $\omega\gamma$, where γ is the clearance rate. This type of formulation best describes the biology as the time to fully clear all parasites increases with more parasites, but each additional parasite increases the overall length at a slower rate, i.e. the marginal effect of each additional parasite on the overall length of infection is decreasing (see Bailey, 1982 for complete derivation). All parasites are assumed to have the same clearance probability regardless of infection date. The frequency of individuals in each state for both the human and mosquito populations was recorded on each day and the recorded dynamics of infection produced statistically rigorous results showing that it has the same dynamics as the classical model that the stochastic IBM is based upon (Figure 2 and Supplementary Figure 1). In addition, at equilibrium, defined in the stochastic case as the distribution of infections after 5,000 days, the distribution of multiply infected individuals in the stochastic case was exactly the same as in the deterministic model.

Because the model has competition between parasite clones inherently built in, it can easily incorporate within-host competition between drug-resistant and drug-sensitive parasites. We assumed that with some probability an infected individual would become infected with resistant parasites, regardless of drug pressure. This simulates the occasional introduction of resistant parasites by individuals traveling to or from other areas where resistance is present. Resistant parasites are assumed to be fully resistant and immutable, and thus treatment is ineffective and their phenotype (including the biological cost of resistance) does not change over the course of the simulation.

To assess the spread of resistance within a population we first let the system come to equilibrium in the absence of resistance, and then introduce the probability of resistance importation. This ensures that resistance will keep appearing at a low rate, so that we can

determine the average time frame for establishment in and spread through the population. We found that once resistance became established it would spread rapidly and every infected individual would harbor drug-resistant parasites within a short time-frame. However, the percentage of the parasite population that was composed of drug-resistant parasites was dependent upon the transmission rate. In our base set of parameters, we assumed an annual entomological inoculation rate (EIR), which is the average number of infectious bites per person per year, of ~25. In this case drug-resistance spread quickly but drug-resistant parasites did not exclude drug-sensitive parasites despite a high rate of drug-treatment (80%) (Figure 3). The same held true for even higher transmission rates, however, at lower transmission levels, drug-resistant parasites were able to exclude the drug-sensitive parasites for the same combination of parameters (Supplementary Figure 2). Thus, in line with prior results examining the impact of superinfection on drug-resistance (Klein et al., 2012), coexistence between drug-sensitive and drug-resistant parasites is a likely outcome, and this effect increases the higher the transmission rate or the greater the fitness cost.

Also in line with prior results (Klein et al., 2012), we find that the long-term viability of drug-resistant parasites is more dependent upon maximizing its duration of infection rather than maximizing the possibility of transmission at any single event (Supplementary Figure 3). Thus, fitness costs that shorten the duration of infection are more likely to be detrimental than fitness costs that reduce the probability of transmission. These results are consistent across transmission rates, however, as the transmission rate increases, the parameter space over which coexistence is possible increases. This suggests that in high-transmission areas, the probability of coexistence between drug-sensitive and drug-resistant parasites is greater. In addition, despite the larger areas of coexistence, it would be easier for resistance to emerge in lower transmission areas, when the cost of clearance is not too great, as resistant parasites with significant fitness costs can still invade, which is also in accord with previous studies (Klein et al., 2008).

Heterogeneous Biting

We first examined how heterogeneous biting impacted the dynamics of the disease in the absence of drug-resistance. The impact of this type of highly skewed distribution is that the equilibrium level of infection in the population is significantly less than in the homogenous biting case despite the same average biting rate (Figure 4), a fact noted in other analyses of heterogeneous biting (Smith et al., 2004). In addition, the distribution of MOIs is skewed, with the majority of infected individuals having an MOI close to one at any one time and a small number of individuals with high MOIs.

Because within-host competition will be reduced in the individuals with lower MOI, we assumed this would impact the evolution of resistance. However, we found that once resistance became established, it generally spread rapidly through the population and reached similar equilibrium levels as in the uniform case. This result belied the rate at which resistance became established though, which was longer when heterogeneous biting was included (Figure 5). However, despite slowing the rate at which resistance becomes established, once it is established, resistance will spread faster in a population with heterogeneous biting than in a population with homogenous biting. These results are qualitatively the same at lower transmission rates, though the difference in waiting time for resistance to establish between the heterogeneous and homogenous biting cases is greater than in the higher transmission case.

Discussion

We developed an individual-based model that is a stochastic analogue to a set of ordinary differential equations. The benefit of this approach is the ability to understand how

deviations from the assumptions inherent within class-based models impact the transmission dynamics. We applied this approach to malaria, the most important parasite species affecting humans. Using a differential equations model based on the Ross-MacDonald framework we examined how two important differences between the models can impact the dynamics of transmission: (1) the role of within-host competition between different parasite phenotypes, in this case drug-resistant and drug-sensitive phenotypes; and (2) differences between individuals, in this case the rate that individuals are exposed to disease.

The emergence of drug-resistance in malaria has been a significant tragedy, resulting in increases in morbidity and mortality (Snow et al., 2001). However, despite the importance of within-host competition in this disease, few models have included this in a manner representative of the dynamics of malaria. In part this is difficult to do in a tractable manner. Using our individual based model, we examined how within-host competition can impact the emergence of drug resistance in a population. We found that as the transmission rate increases, the range over which drug-resistant parasites can exclude drug-sensitive parasites contracts significantly, while the range over which coexistence can occur increases (Supplementary Figure 3). In particular, while prior analyses (Klein et al., 2012) found the parameter range over which the system transitions from all sensitive to all resistance to be fairly sharp, our new model finds that this is actually a fairly broad range, particularly at higher transmission levels. This suggests that a population of drug-resistant parasites could encompass parasites with a range of fitness costs, making it easier for parasites to either acquire step-wise resistance mutations (Hastings and Watkins, 2006) or compensatory mutations (Jiang et al., 2008).

Emergence of antimalarial drug-resistance has historically occurred in low transmission areas (White, 1998; White and Pongtavornpinyo, 2003). There are a number of theoretical suggestions as to why that has been the case: (1) within-host competition in higher transmission areas reduces the frequency of resistant alleles (Hastings, 1997); (2) immunity, which reduces the rate that parasites see drug treatment (Hastings, 1997; Plowe et al., 1998; White, 1998) creating a refuge for drug-sensitive parasites (Klein et al., 2008), and also reduces the probability of survival for resistant parasites (Gatton et al., 2003; Plowe et al., 1998); and (3) higher selfing rates in low transmission areas reduces the likelihood that resistance encoded by mutations at multiple loci will be broken up (Dye and Williams, 1997; Hastings, 1997; Paul et al., 1995). However, most of these theories deal with the reasons that resistance is more likely to appear *de novo* in low transmission areas, and not with the role of establishment.

Establishment of resistance in a population is important, because once resistance becomes established it becomes more difficult to use control methods to eliminate resistance. Our results found that while establishment occurred faster, on average, in high transmission settings, heterogeneous biting played an even more significant role than transmission. Heterogeneity in the probability of being bitten by a mosquito impacts establishment because when resistance is introduced into the population, the resistant parasite will either be in an individual with a high probability of being bitten, and thus would face tremendous competition, or it will be in an individual with little competition but has a low chance of onward transmission because these individuals are bitten infrequently.

Once established, the resistant parasites will spread at a rate that is governed by the biological fitness costs of resistance, drug treatment rates, as well as the transmission rate, which impacts individual MOI and within-host competition (White, 1999b). We found that holding drug treatment rates constant, the rate that resistance increased from its establishment level is faster when heterogeneous biting occurs regardless of transmission rate. This is because once resistance becomes established, (a) individuals with low

probabilities of being bitten by a mosquito are less likely to get reinfected with sensitive parasites when they are infected which generates a reservoir of resistance, and (b) individuals with higher transmission rates will quickly spread resistant parasites due to their high biting rates. This point is distinct from other models discussing the role of transmission in the initial emergence of resistance which have generally suggested that resistance is more likely to emerge in low transmission areas. Instead it is a reflection of the role that large numbers of individuals with low MOIs combined with a small core of individuals responsible for the majority of transmission play in speeding resistance through an area, an idea that is akin to the spread of sexually transmitted diseases (Hethcote and Yorke, 1984). Thus, in areas with highly variable transmission patterns, reducing transmission in the “core” group responsible for the majority of transmission is essential to slowing the spread of resistance.

Here we have introduced a computationally efficient individual based model for examining infectious diseases. The model was applied to malaria and the impact of within-host competition and heterogeneous biting on the emergence and spread of drug-resistance. The model results have shown themselves to be extremely computationally efficient and provided statistically rigorous results that conformed with the underlying deterministic model. This approach allowed us to examine how assumptions of the deterministic equations were impacted by individual differences. The results clearly show that within-host competition is important for understanding the emergence and spread of drug-resistance, and provided more robust predictions as to how this interacts with transmission intensity. Future extensions to this point will consider the role of immunity, which has been shown to be an important component in the evolution of antimalarial drug resistance (Klein et al., 2008).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- We embedded within-host competition in an agent-based model of malaria
- We examined the role of heterogeneous biting on the establishment and spread of drug resistance
- We find that heterogeneous transmission slows the establishment of resistance in a population
- We find that heterogeneous transmission speeds the spread of resistance once established

Figure 1. Schematic of Deterministic Model

S refers to susceptible individuals, I_i is individuals infected with i genetically distinct clones, and M_S , M_L , M_I refer to susceptible, latent and infectious mosquitoes, respectively.

Figure 2. Comparison of individual based stochastic model with classical formulation of multiple infection malaria model

A) The dynamics of the classical model are described by the solid black lines, while one standard deviation of the mean of 10,000 simulations of the individual based model is overlaid. B) The frequency of the population with a specific MOI was estimated at the end of the simulation. The distribution of MOIs that result from the classical model is described by the grey bars, and one standard deviation of the mean of 10,000 simulations of the individual based model is overlaid.

Figure 3. Antimalarial Drug Resistance Evolution in Mixed Infections

As drug-resistance spreads through the population, the impact of treatment in reducing the burden of infection is negated (difference between dotted red line and dashed grey line). However, despite the percentage of the population infected with at least one resistant parasite quickly reaching 100%, the percentage of parasites in the population that are resistant (blue line) increases at a slower rate, and in fact never goes to 100%, despite the continual introduction of resistance de novo (red dots). Annual EIR was ~25 and drug-resistant parasites were assumed to have fitness costs of 32% and 17% for transmission and clearance, respectively.

Figure 4. Impact of Heterogeneous Biting on Transmission and Multiplicity of Infection Heterogeneous biting has a significant impact on the transmission dynamics of malaria (A). Because the rate of biting on a large proportion of the population is significantly lower, the level of infection in the community is lower. The gray lines are the dynamics of the population in the absence of heterogeneity in biting (dashed is infected individuals and solid is susceptible individuals). The black lines represent the mean of 500 simulations of the individual based model, and one standard deviation of the mean is overlaid. Heterogeneous biting also significantly impacts the number of individuals that are multiply infected (B). Because we assumed that only 20% of individuals got 80% of the bites, only a small proportion of the population have high levels of multiple infections, while the majority of the population has only a small number of infections. The black lines are the mean of 500 simulations of the individual based model, and one standard deviation of the mean is overlaid.

Figure 5. Impact of Heterogeneous Biting on the Establishment and Spread of Resistance

Because resistance was introduced stochastically, we simulated the model 2,000 times and measured the time until resistance became established, which we defined as reaching 0.01% with and without heterogeneous biting at two different transmission rates (in A EIR ~25 and in B EIR ~6). We then measured the time from establishment till resistance reached 0.05%, 0.1%, 0.5%, 1%, 5%, 10% and 25%. In all cases the fitness costs of transmission and clearance were 32% and 17%, respectively. In the heterogeneous case, because a small group of individuals receive the vast majority of bites, resistant parasites that are introduced either face higher levels of within-host competition or are in individuals that don't get bitten much. Thus, on average, the time until resistance became established in the population is slower than the homogenous biting case (blue bars). However, once resistance becomes established, it will spread through a population with heterogeneous biting faster, as the majority of individuals have significantly less within-host competition. The green bars measure the time for resistance to increase from 0.01% to each resistance frequency. Resistance takes a longer time to establish in heterogeneous case, but spread occurs at a faster rate when biting is not equally distributed. Error bars are 95% confidence intervals, and black horizontal lines are the median values.

Table 1

Parameters Used in the Model

Parameter	Value	Source
g Mosquito life span	10 days ⁻¹	(Macdonald, 1957)
n Days to sporogony	10 days ⁻¹	(Beier and Vanderberg, 1998; Pringle, 1965; Rosenberg and Runsiwongse, 1991)
a Human biting rate	0.3 days ⁻¹	(Saul et al., 1990)
m Mosquitos per person	Variable	(Killeen et al., 2000; Macdonald, 1957)
b Transmission efficiency from mosquitoes to humans	0.5	(Macdonald, 1957)
c Transmission efficiency from humans to mosquitoes	0.625	(Saul et al., 1990)
ψ Rate that clinical symptoms arise	0.004 days ⁻¹	Author Estimate
ρ Fraction of symptomatic patients that use drugs and successfully clear sensitive parasites	0.80	Author Estimate
μ Average human lifespan	45 years ⁻¹	WHO
γ Recovery rate from an infection	200 days ⁻¹	(Eyles and Young, 1951; Smith et al., 2005)
δ Disease induced death rate	0.0001 days ⁻¹	Author Estimate
R Rate of resistance importation	2.0/year	Author Estimate