Population biological principles of drug-resistance evolution in infectious diseases

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The emergence of resistant pathogens in response to selection pressure by drugs and their possible disappearance when drug use is discontinued are evolutionary processes common to many pathogens. Population biological models have been used to study the dynamics of resistance in viruses, bacteria, and eukaryotic microparasites both at the level of the individual treated host and of the treated host population. Despite the existence of generic features that underlie such evolutionary dynamics, different conclusions have been reached about the key factors affecting the rate of resistance evolution and how to best use drugs to minimise the risk of generating high levels of resistance. Improved understanding of generic versus specific population biological aspects will help to translate results between different studies, and allow development of a more rational basis for sustainable drug use than exists at present.

Introduction

The history of efforts to control infectious pathogens shows one disconcerting fact: wherever antimicrobial drugs have been used for prolonged periods, resistant pathogens have evolved. Resistance evolution has seriously undermined our ability to control many important diseases, with substantial economic and public-health costs.[1,2] Only 6 years after the introduction of the first antibiotic (penicillin) in 1943, about 60% of *Staphylococcus aureus* isolates obtained from British hospitals were penicillin-resistant.[3] Efforts to control malaria have shown the same pattern—the enormous success of chloroquine was progressively undermined by widespread resistance.[4]

By comparison with the tremendous financial and scientific resources directed towards the development of new antimicrobials, little is done to investigate how to best prolong the usefulness of available drugs. A clear understanding of how to prolong the usefulness of drugs not only requires cost-intensive clinical studies, but also an improved understanding of the main factors underlying the evolutionary dynamics of pathogen resistance. Mathematical and computational models of population biology and evolutionary dynamics of infectious pathogens during treatment play a central part in uncovering these factors.

The rise and fall of drug resistance are evolutionary processes characterised by competition between resistant and sensitive pathogen strains. These processes can occur in individual patients or in host populations. Accordingly, medical practitioners face two challenges in the context of resistance: management of transmitted resistance and prevention of de-novo resistance. Any long-term strategy to counter resistance must also deal with de-novo resistance, because it must at some point have arisen de novo, either by mutation or by horizontal gene transfer.

A large body of evidence has accumulated over the years, which describes the evolutionary dynamics of resistance at the within-host or between-host level and for different diseases or drugs. The evolutionary dynamics at these host levels and processes of resistance for different drug–pathogen combinations share many similarities, despite vast differences in underlying genetic and molecular mechanisms. In this Review, we aim to synthesise published works about the evolution of resistance in infectious diseases and establish which processes are generic and which are specific to the drug, the pathogen, or the within-host or between-host level.

Emergence of resistance within host

The dynamics of resistance mutations in the presence of drugs depend crucially on whether these mutations already exist at appreciable levels in the population at the timepoint when the pathogen population is first exposed. Although drug-resistance mutations are costly to pathogens in many cases, they might exist in drug-naive pathogen populations, either because they are continuously generated by mutation in sensitive strains or because they immigrate from outside sources. The frequency of these mutations in untreated populations is thus determined by a balance of selection, mutation, and immigration: given as $\mu_{s\to r}/s_A$ and m_r/s_A, where $\mu_{s\to r}$ is the mutation rate from the sensitive to the resistant strain, m_r is the immigration rate of resistant pathogens, and s_A is the difference in fitness between sensitive and resistant strains in the absence of drugs. More complicated expressions are obtained if more than a single mutation is needed to confer resistance.[5] Thus, mutation and immigration can lead to appreciable levels of drug-resistance mutations in pathogen populations not exposed to drugs, particularly when selection against these mutations is weak.

If no drug-resistance mutations exist before treatment initiation, then two dynamic phases precede emergence of resistance. First, the mutations must emerge in the focal population (figure 1A), which is a chance event most appropriately described by a stochastic process. The probability that a resistant mutant occurs at a given time window increases with the immigration or mutation rate. If resistant pathogens are generated by mutation, the probability of drug-resistance mutations increases

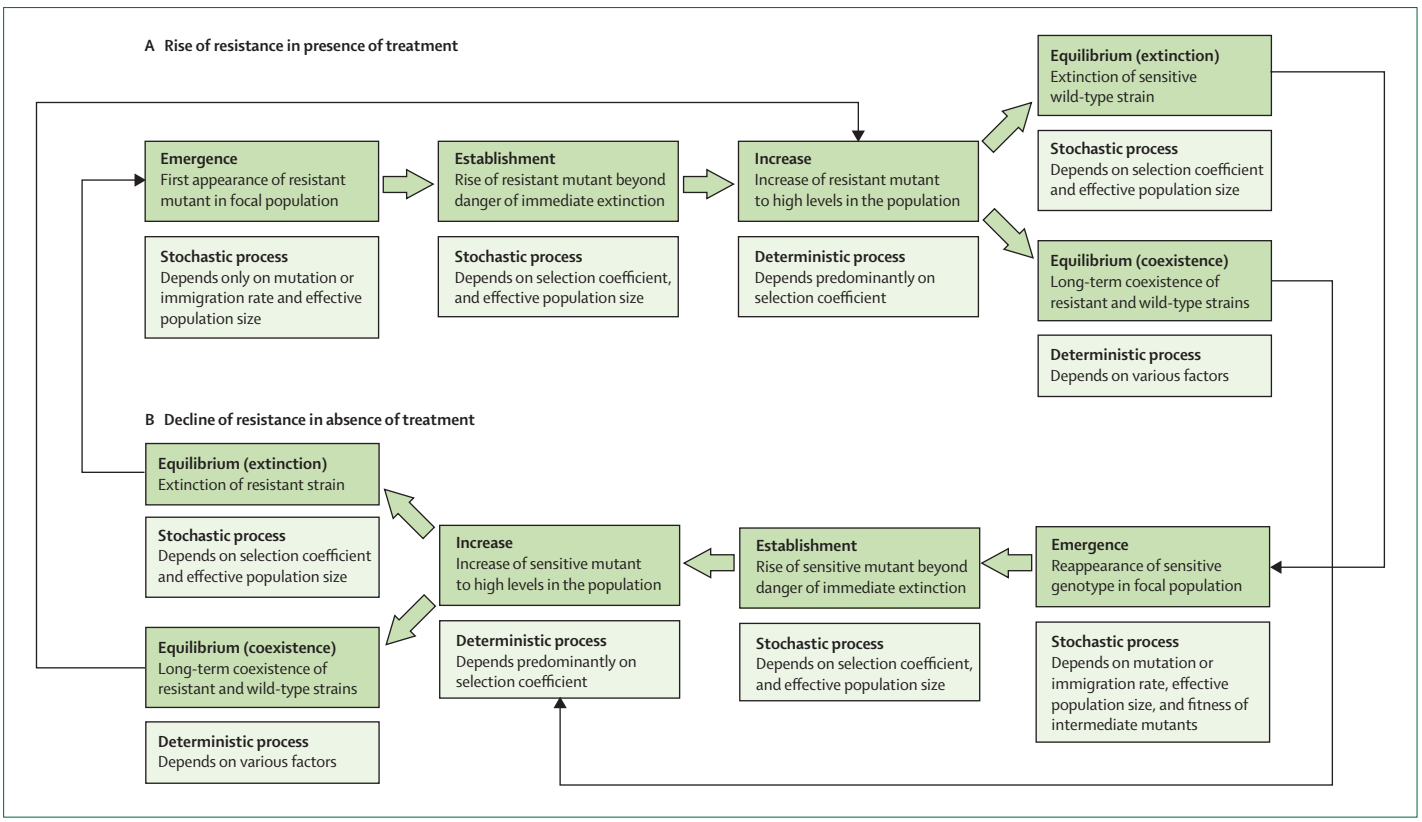

Figure 1: **Evolutionary dynamics of resistance in the presence and absence of drug selection**
The rise (A) and fall (B) of resistance are characterised by the appearance and competition between resistant and sensitive strains of the pathogen, and the evolutionary process can usefully be subdivided into several related dynamical phases.

with the population size, N; table 1 shows examples of population sizes. The first—potentially transient—appearance of a resistance mutation does not, however, depend on the selection coefficient in presence of drugs. The probability that no resistant mutant appears over a period of t generations is $(1–\mu_{s\rightarrow r})^{Nt}$, which gives an expected time to appearance of the first mutant of about $1/(\mu_{s\rightarrow r}N)$ when $\mu_{s\rightarrow r}$ is small. In fact, the frequency of resistant mutants is often used to infer mutation rates in bacteria.[58]

Once the first resistant strain has appeared in the focal population, it is subject to selection by drugs, depending on specific pharmacodynamics and pharmacokinetics (tables 2–4). The establishment phase (figure 1A) then follows, with possible increases in the number of resistant strains, from a single or very few copies to a sufficiently large number, such that extinction for stochastic reasons becomes improbable. Pathogens are often characterised as resistant if they have a growth advantage over the sensitive wild type in the presence of drugs. This advantage can be expressed by a selection coefficient (s_P). When only a few copies of a resistant strain exist, the probability of it avoiding extinction increases with s_P and the number of copies already present. A classic result from population genetics is that the probability of fixation of a single advantageous mutant in a large population is:[99,100]

$$1–e^{-2s_PN_e/N}$$

Here N_e is the effective population size of a theoretical population with the same degree of stochastic effects as the population under investigation. N_e is notoriously difficult to determine,[7] but typically is much smaller than N.[101] Progress in the estimation of fixation probability of beneficial mutations is reviewed by Patwa and Wahl.[102] However, a fitness advantage over the drug-sensitive wild type is not sufficient, because a strain that is fitter than the wild type in the presence of drugs might still not be fit enough to survive. To illustrate the stochastic nature underlying the dynamics of resistance, we show some simulation results in the webappendix.

See Online for webappendix

Once the resistant strain has grown to a population size such that its extinction has become improbable, further population growth is governed mainly by selection, and can be described as a deterministic process (figure 1A). Roughly, the time until a resistant strain dominates the population is proportional to $1/s_P$ and decreases with the logarithm of factors such as the mutation rate ($\mu_{s\rightarrow r}$) or the initial frequency of resistant

	HIV	Influenza	*Mycobacterium tuberculosis*	*Staphylococcus aureus*	*Plasmodium falciparum*
Population size (number of cells or pathogens/host)	Number of infected cells, 10^7–10^8 (ref 6); effective population size disputed but probably much smaller (ref 7)	Estimated target cell population, 4×10^8 (ref 8), but reliable estimates difficult to obtain	10^8–10^9 bacteria per lesion (ref 9)	In bacteraemia, diverging estimates depending on method: 5×10^7 (colony forming unit [ref 10]) to 4×10^{10} (PCR [ref 11])	Symptom onset in non-immune individuals, 10^8–10^9; lethal malaria, 10^{12}–10^{13} (ref 12)
Genetic mechanism of resistance acquisition and their rates*					
Mutation	μ is $3{\cdot}5\times10^{-5}$ (ref 13); number of base-pair substitutions necessary for resistance varies strongly (resistance to lamivudine only one [ref 14], high-level resistance to tipranavir up to six mutations [ref 15])	$\mu\approx10^{-5}$/replication (ref 8); eg, amantidine-resistance, $f_r\approx10^{-4}$ (ref 16)	Eg, isoniazid-resistance, $f_r\approx10^{-6}$–10^{-8} (ref 17)	Eg, vancomycin-resistance (ref 18); $\mu\approx10^{-6}$–10^{-10}/replication (ref 19)	Eg, atovaquone-resistance (ref 20); $\mu_r\approx10^{-11}$–10^{-20}/replication in vivo, higher in vitro (refs 12,20)
Recombination	Frequent recombination, but probably minor effect on evolution of resistance	Frequent reassortment (refs 21,22), but homologous recombination might be rare (refs 23,24)	..	..	During the sexual stage of mosquitoes life cycle, recombination is very frequent (ref 25), but population biological consequences unclear (ref 26)
Horizontal gene transfer	..	..	Horizontally transmitted resistance unknown (ref 17); horizontal gene transfer extremely rare (ref 27)	Generally, gene alteration six times more likely by gene transfer than by mutation (ref 28); eg, vancomycin resistance (refs 18,29)	No evidence for horizontal gene transfer (ref 30)
Molecular mechanisms of resistance (indicated whether mechanism plays a part in disease and examples given)					
Alteration, circumvention, or overexpression of target protein	Target alteration (ref 31)	Target alteration (ref 16)	Eg, isoniazid resistance (ref 17)	Eg, vancomycin resistance (ref 18)	Eg, atovaquone resistance (ref 20), additional examples given by Uhlemann and colleagues (ref 32)
Degradation of drug	..	..	Intrinsic resistance partly conferred by β-lactamase (ref 33)	Eg, β-lactamases (ref 18)	No reports found
Efflux pumps or changing permeability	..	..	Efflux pumps identified (ref 34), but seem to play minor part in clinical resistance	Eg, quinolone resistance (ref 35)	Eg, mefloquine resistance (ref 36)
De-novo *vs* transmitted resistance	Both, however, up to now only low levels of transmitted resistance (ref 37)	Both, levels of primary resistance increased in seasonal influenza (ref 38), first transmission of resistant virus in pandemic influenza (ref 39)	Resistance acquisition during monotherapy very common, (35 of 41 patients in 6 months [ref 40]), but primary resistance in up to 14% of infections and up to 94% of multidrug-resistant infections [refs 41–43])	Often primary (ref 44), sometimes acquired (ref 45)	Most often primary (ref 46), sometimes acquired (ref 47)
Mode of transmission	Sexual transmission, intravenous drug use, or mother to child	Airborne, contaminated surfaces (ref 48)	Airborne (ref 49)	Health-care workers as vectors (ref 50), contaminated surfaces (ref 51)	Mosquitoes as vectors (ref 52)
Recommended treatment strategies and duration	Lifelong combination therapy (ref 53)	Treatment course of 5 days, combination discussed by Glezen and colleagues (ref 54), and Wu and colleagues (ref 55)	Combination therapy with (initially) at least four drugs—duration of therapy for non-multidrug-resistant disease, 6–18 months, and for multidrug-resistant tuberculosis, 2 years (ref 42)	For bacteraemia, at least 14–days' treatment with glycopeptides or linezolid (ref 56)	3–7 day combination therapy with two drugs (ref 57)

Further factors potentially affecting resistance dynamics that are not listed here are the fraction of treated hosts, slowly replicating populations (ie, persisters or latent stages), and generation time (ie, time between primary and secondary cases). Ref=reference. *Examples and rates of acquisition are given if known; three different measures of rates of resistance acquisition are given, depending on which data are available. The most straightforward measure is the rate of acquiring resistance per replication (μ_r). This rate depends on the number of possibilities to acquire resistance (r), number of mutations needed for resistance (n), and mutation rate per base pair and replication (μ). Because μ_r often cannot be determined directly, the frequency of resistant mutants (f_r) in a drug-naive population is used. This measure, however, depends on μ_r and the fitness costs of mutations.

Table 1: **Overview of factors that could influence resistance evolution for various diseases**

mutants at the start of the deterministic phase.[103,104] Hence, in accordance with experimental studies,[105] minor differences in the selection coefficient (s_P) play an important part in the emergence rate of resistance, whereas the mutation rate or the initial frequency of resistant pathogens need to change over orders of magnitude to have an appreciable effect.

Eventually, a resistant strain might converge towards an equilibrium frequency in the presence of drugs (figure 1A). Such a strain might drive the sensitive strain to extinction or coexist with the sensitive strain in the face of continued drug-selection pressure. Complete extinction of the sensitive strain can be prevented by back-mutation, immigration of sensitive

strains, drug-free sanctuary sites, or if the fitness advantage of the resistant strain decreases as its frequency increases in the population (ie, negative frequency-dependent selection).[106]

Continued evolution of resistance

After the emergence of a drug-resistance mutation, other mutations can appear that further increase the fitness of their carrier under drug-selection pressure. We discuss two types: additional drug-resistance mutations and compensatory mutations. The basic dynamics of additional mutations are the same as those for the first resistance mutation (figure 1A). However, an important complication is that the fate of a new mutation can depend on whether it arises in a genotype that already carries a drug-resistance mutation, or in a wild-type organism. The first resistance mutation to emerge and establish in a population under drug-selection pressure is often neither the only one to arise nor the one that yields the fittest genotype. In the simplest case, a second resistance mutation increases the efficiency of the first in an additive way (figure 2A), so that the genotype combining both mutations will spread in the population. However, if both mutations arise in the wild-type genotype and are not combined by recombinational processes, they will compete with each other and one will eventually drive the other to extinction, a process known as clonal interference.[107]

More complicated situations can occur if there are epistatic fitness interactions between the two mutations. For example, on the one hand both mutations can confer substantial levels of drug resistance only when combined, but not alone (positive epistasis; figure 2B). On the other hand, the two mutations might improve fitness when on their own, but impede each other or have synergistic costs such that the genotype carrying both mutations has a lower fitness than either of the single mutants (negative-sign epistasis; figure 2C). The order in which the two mutations arise in the population can be decisive—after establishment of one mutation, the second is unlikely to arise in a wild-type genotype and will therefore be selected against.

Fitness costs are commonly associated with drug-resistance mutation.[108–110] Hence, after the establishment of resistance mutations, compensatory mutations, that ameliorate the fitness costs, might be selected for. Compensatory mutations have been reported in various organisms, including bacteria,[111,112] fungi,[113,114] and HIV.[115] By definition, compensatory mutations increase the fitness of an organism carrying a drug-resistance mutation, but are deleterious (or at best neutral) in a wild-type organism (figure 2D). Therefore, compensatory mutations are expected to establish only once resistance mutations are sufficiently common in the population. Compensatory mutations can reduce the fitness costs of resistance either in the absence or in the presence of drugs or in both situations.

	Explanation and theoretical references
Timing	
Treatment course as long as necessary[59]	Apart from the danger of relapse,[60] treatment courses that are too short can lead to increased numbers of resistant or partly resistant pathogens, or with combination treatment to increased numbers of pathogens that are resistant to one of the drugs used[61]
Treatment course as short as possible[62]	R_0, the number of secondary infections a single carrier causes in a completely susceptible population, of resistant strain rises with the duration of treatment[63]
Initiation of treatment when pathogen population is small[64]	Treatment initiation when the pathogen population is still small might limit emergence because the probability of resistant mutants emerging is low[60,63,65,66]
Ensure waiting time after last use of same drug is long enough[67]	Both for plasmid-borne[68] and for chromosomal resistance,[66] the likely success of subsequent treatment courses might increase with waiting time, because the fraction of resistant pathogens could decline
Drug dose	
High drug concentration[62]	For chromosomal resistance, suboptimum treatment might allow outgrowth of resistant pathogens with more costly or less efficient (intermediate) resistance mutations.[66,69–71] These intermediate mutations can then give rise to fully resistant organisms. Even if the resistance mutation was not present when treatment was started, suboptimum treatment might allow replication of the parental sensitive strain and simultaneously select for resistant mutants.[72] Acquisition of plasmid-borne resistance is also facilitated by suboptimum treatment because low antimicrobial concentrations often have only bacteriostatic effects (dormant cells can still receive plasmids[68])
Optimisation of dose regimen[73–75]	If the effectiveness of the antimicrobial saturates at a certain concentration, infrequent high doses will always be less effective than frequent lower doses.[76] However, in a less susceptible subpopulation, high peak concentrations might prevent an outgrowth of these pathogens.[77] Nevertheless, minimisation of the time in which the effective antimicrobial concentration inhibits growth of wild-type cells, while allowing replication of resistant organisms is important[70,78]
Adherence[79]	Non-adherence can lead to an increase in the number of resistant pathogens[80] or allow outgrowth of intermediate resistant pathogens,[81] especially when doses are missed early in treatment[60]

References in the first column are clinical or experimental studies related to the strategies mentioned.

***Table 2*: Overview of within-host treatment strategies and explanations for why they could reduce, prevent, or delay resistance**

	Explanation and theoretical references
Prophylaxis[82,83]	The efficiency of prophylaxis depends on the size of the treated population and on the probability of emergence of resistance. Early and widespread prophylaxis may lead to eradication of the resistant strain and thus containment of the disease. If this is not successful, prophylaxis may even exert a stronger selection pressure than treatment, because susceptible individuals can only be infected by resistant strains[84]
Combination therapy (standard for tuberculosis, HIV, and malaria)	If resistance to two drugs is acquired independently, combination therapy decreases the probability of de-novo acquisition of resistance, because a pathogen with two appropriate mutations is unlikely to exist in the initial population.[80] However, heterogeneous drug concentrations could allow acquisition of single and then double resistance.[85] In the case of resistance genes located on incompatible plasmids, combination therapy might lead to recombination of these two genes on one plasmid and thus transferable multiple resistance[86]
Limitation of drug treatment to necessary cases[87,88]	Antibiotic consumption and resistance emergence, or resistance spread, or both emergence and spread of resistant strains are positively correlated.[89–91] Therefore, reduction of consumption by only treating when necessary will slow down emergence of resistance
Appropriate treatment of resistant infections	Drug-susceptibility testing and appropriate treatment should lead to reduced transmission of resistant strains of tuberculosis, and to reduced mortality[92]

References in the first column are clinical or experimental studies related to the strategies mentioned.

***Table 3*: Overview of within-host and between-host treatment strategies and explanations for why they could reduce, prevent, or delay resistance**

	Explanation and theoretical references
Minimisation of transmission (eg, reduction of hospital overcrowding[93])	Minimisation of transmission in a hospital will have a larger effect on resistant infections than on sensitive cases, since incoming patients are more likely to carry sensitive pathogens[94]
Structuring monotherapy in host population by random assignment to one of the drugs (mixing) and scheduled changes of formulary for whole population (cycling)[95,96]	On the population level, mixing could lead to more multiresistance but less resistant cases in total than cycling,[61,97] because individual bacterial strains face more frequent environmental changes with this strategy.[61] Also, cycling periodically reduces the mean fitness of the parasite population more quickly than combination therapy, thereby allowing the invasion and spread of new resistant types[98]

References in the first column are clinical or experimental studies related to the strategies mentioned.

Table 4: **Overview of between-host treatment strategies and explanations for why they could reduce, prevent, or delay resistance**

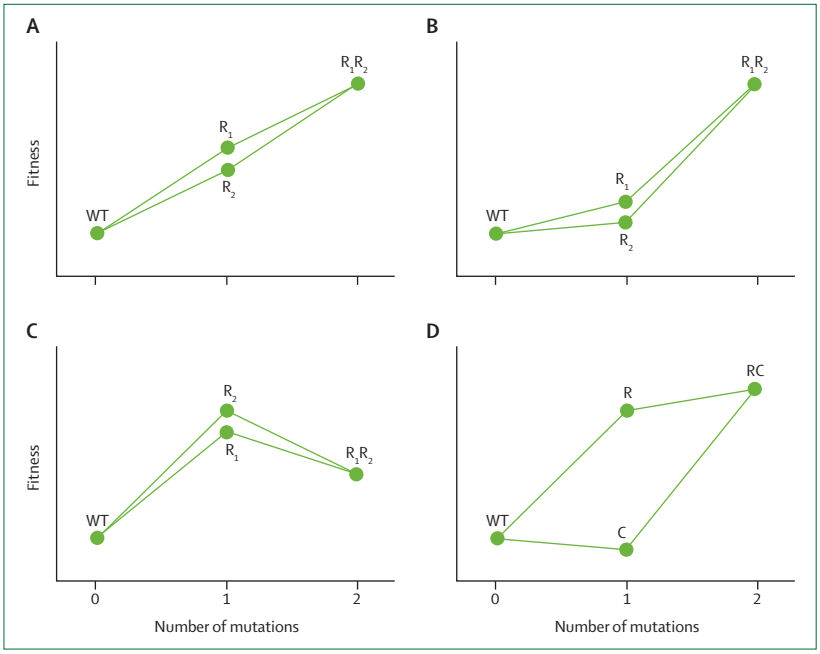

Figure 2: **Simple two-locus fitness landscapes for resistance and compensatory mutations**
Additive effect of two resistance mutations (A). Epistatic interaction in which substantial resistance is only achieved if both mutations are present (B). Epistatic interactions in which the presence of both resistance mutations decreases fitness (C). Resistance with a compensatory mutation (D). WT=wild-type genotype. R=mutation conferring resistance. C=compensatory mutation.

When several mutations are needed for resistance to be clinically significant, the most important issue is the fitness of intermediate mutants in the presence of antimicrobials that select for resistance. Fitness landscapes can be more complex than the simple two-locus examples given above. A case in point is the evolution of resistance to the β-lactam antibiotic cefotaxime for treatment of *Escherichia coli* infections,[116] in which five point mutations are needed to produce full resistance. However, only a few of the mutational pathways leading to the fully resistant genotype are selectively accessible, and most involve neutral or even deleterious mutational transitions. Together with a recent study of evolutionary trajectories of drug-resistance mutations,[117] this finding suggests that epistatic interactions between different mutations can have a great effect on the likelihood and rate of resistance evolution.

Reversion in absence of drugs

Conceptually, the disappearance of drug-resistance mutations after drug-selection pressure is withdrawn strongly resembles the emergence of these mutations in the presence of drugs (figure 1). If the sensitive strain has become extinct during drug selection, the reversal to drug sensitivity has to begin by reintroduction of the sensitive strains either through back-mutation or immigration (figure 1B).

After the sensitive wild type has reappeared, its population first needs to grow such that extinction for stochastic reasons becomes improbable (figure 1B). The probability that sensitive strains emerge in this phase with sufficient frequency increases with their selective advantage in absence of drugs (s_A) and with the effective population size. Once sensitive pathogens have a sufficiently large population, the dynamics that follow can be described deterministically (figure 1B). Importantly, the selective advantage of sensitive over resistant strains in absence of drugs (s_A), is typically much smaller than that of resistant over sensitive strains in presence of drugs (s_P). Consequently, the probability of a population increase from a small population and the rate of increase in the deterministic phase are substantially larger for resistant strains under drug-selection pressure than for sensitive strains in the absence of drugs. However, s_P depends on intensity and (population-wide) coverage of treatment. For weak treatment (low s_P) or strong competition, rise and fall of resistance might be similarly fast, as reported for chloroquine-resistant malaria in Malawi.[118] But unfortunately, general estimates for the rate of emergence and decline of resistance are difficult to make. HIV is nearly always treated in developed countries, but not necessarily in resource-limited settings. *S aureus* is commonly seen in the microflora, but only treated when it becomes invasive. Furthermore, a drug used to treat one infection can also select for resistance in other pathogens.

In the long term, disappearance of resistant strains in the absence of drugs depends on several factors (figure 1B). If resistant strains have acquired compensatory mutations that increase fitness in absence of drugs, reversion back to sensitivity is more unlikely or even impossible because the mutational pathway back might go through a point of prohibitively low fitness (a so-called fitness valley).[66,119,120] Moreover, resistance could also be maintained by mutation or immigration. Sanctuary sites, where pathogens might not be in direct competition with sensitive strains or might persist because of reduced or absent population turnover,[121,122] can lead to long-term persistence of resistance even when drug-selection pressure is discontinued. Finally, resistance genes might be linked

to others under selection, which increases their probability of being maintained.

Mechanisms of resistance acquisition

Despite general patterns in evolution of resistance, underlying genetic and molecular mechanisms strongly differ between individual pathogens. Resistance can be acquired either by mutation, recombination, or horizontal gene transfer. Although resistance can be acquired by mutation in all diseases discussed in this Review, the ability of horizontal gene transfer differs tremendously between pathogens (table 1). For example, resistance in *Mycobacterium tuberculosis* usually results from point mutations, whereas many other bacterial pathogens can acquire resistance by horizontal transfer.

Mutation rates vary widely—for example, the mutation rate of influenza can be several orders of magnitude higher than that of malaria (table 1). The importance of mutation rates is uncontroversial,[66,80,103,104,123,124] and associations between resistance and high mutation rates have been shown.[125] Although hypermutators seem to accelerate resistance emergence in cystic fibrosis,[126] very high mutation rates can also cause accumulation of deleterious mutations. Treatment failure might therefore be most likely at intermediate mutation rates when resistance is generated with sufficient frequency, but the fitness costs of hypermutability are still low enough to allow survival.[127]

Homologous recombination, common in many pathogens (table 1), cannot generate new resistance mutations but can combine those that have originated in different genomes. However, recombination need not accelerate the emergence of resistance, because it can also break up combinations of resistance alleles. Mathematical modelling has shown that recombination accelerates adaptation only under certain circumstances (negative epistasis and intermediate strength of stochastic effects).[128] For within-host evolution of HIV, these circumstances are unlikely to be met.[129–133] The extent to which these findings apply to other pathogens (eg, malaria; table 1) is unknown, but the results obtained for HIV suggest that homologous recombination is only a minor force in pathogenic adaptation to drug resistance.

In many bacteria, genetic material can be transferred between species via horizontal gene transfer (table 1),[134] examples of which are the emergent vancomycin resistant *S aureus*[28] or the spread of the resistance gene *NDM-1*.[135] Plasmids often contain several resistance genes, so that one drug can select for multiresistance.[136] Rapid spread of plasmids through bacterial populations enables them to escape negative selection by moving to other hosts.[137] Although the dynamics of plasmid-borne resistance and mutational resistance might therefore differ, these differences are still largely unexplored.

Individual patients and populations of patients

The importance of the within-host versus between-hosts level varies strongly between pathogens (table 1). In some chronic diseases, such as tuberculosis, the rate of resistance acquisition during treatment is substantial (as long as a substantial proportion of infections are still susceptible). De-novo acquisition of resistance might be less important in other infectious diseases, such as malaria, in which resistant strains are mostly acquired.

Full characterisation of epidemic spread of resistance would need to describe the pathogen dynamics at both the within-host and between-host levels[46] and can be achieved by so-called nested models.[138]

For practical and conceptual reasons, however, ignoring the within-host dynamics and classifying hosts according to whether they are infected with a given strain or not is often useful. A consequence of this shortcut is that not all population dynamic and population genetic insights that apply to the within-host level apply in the same way to the between-host level. For instance, mutation at the within-host level describes a stochastic process by which sensitive cells give rise to resistant mutants (or vice versa). The equivalent process at the between-host level is transformation of drug-sensitive infection to drug-resistant infection; this is not purely stochastic, but a combined process of mutation and selection. The process can therefore be either mostly stochastic for small within-host populations or an almost deterministic switch for large within-host populations.

Both the between-host and within-host level are characterised by their specific heterogeneities and population structures, which often affect the evolution of resistance. At the within-host level there are two types of heterogeneities: temporal heterogeneities, which are caused by imperfect adherence or pharmacokinetic effects, and spatial heterogeneities, which are caused by differences in tissue penetration. Such heterogeneities have been shown both in theoretical model and in animal experiments to facilitate resistance evolution.[72–75,139] Indeed, bacterial populations can show a remarkably heterogeneous degree of resistance.[140,141] However, this heterogeneity could also, at least in part, be explained by specific molecular mechanisms of resistance (table 1).

If resistance is caused by drug-degrading enzymes, presence of resistant cells could lead to population-wide protection and therefore allow persistence of susceptible pathogens. A special form of heterogeneity occurs if a fraction of the pathogen population remains in a dormant form, which confers phenotypic resistance to antimicrobial therapy. Examples include latently infected cells in viral infections such as HIV or persisters in bacteria.[121,122] This phenotypic resistance might promote the evolution of drug resistance by prolonging treatment (eg, latently infected cells are the reason why lifelong treatment of HIV is needed), but also directly by generating a protected compartment, which can serve as a source of drug-resistance mutations.[142] At the between-host level, clinical and theoretical studies have shown that population structure can affect the spread of resistance. Important examples of the effect of population

structure at the epidemic level include the interaction between community and health-care facilities,[143–145] treated and untreated hosts,[146] human and animal populations,[147] and asymptomatic carriers and infected patients.[145] Findings from several studies suggest that a heterogeneous environment, generated by the use of different antibiotics, could be used as a weapon against the spread of resistance.[63,97]

One of the most prominent factors that distinguish the within-host from between-host levels is the way the immune system interferes with resistance evolution. Such immune interference could substantially impede the evolution of drug resistance for bacteria[148] and viruses.[149] Specifically, additional killing of pathogens by the immune system might narrow the conditions under which resistance emerges.[66,148] Immunity has been suggested to counteract resistance, but since sanctuary sites facilitate resistance, abscess formation might have the opposite effect.[150] Furthermore, immunity might also indirectly affect the epidemic spread of drug resistance.[151] For instance, individuals that are immune to plasmodium could serve as a refuge for drug sensitive strains, because they tend to be asymptomatically infected and hence are less likely to use antimalarial drugs.[152] Another peculiarity of the within-host level is the role of commensal microflora, which is a major source of resistance mutations in many bacterial diseases.[153]

Treatment strategies and control

Background

Meaningful criteria are required to assess the success of treatment strategies. An aim to reduce resistance by itself is meaningless, because this could be trivially achieved by complete discontinuation of drug use.[97] Useful measures need to combine aspects of disease burden,[84] cost-effectiveness,[154] and lifespan of drug effectiveness. Selection for resistance can be minimised by limiting drug use to necessary cases, reducing transmission, or reducing the probability of resistance emergence per treatment course (tables 2–4). For individual patients, the aim of antimicrobial therapy typically is to eradicate the pathogen or at least to stop its replication, therefore preventing the rise of drug-resistance mutations. At the epidemic level, the rise of resistance is unavoidable for most pathogens, therefore control efforts focus on delaying and minimising resistance. Potential trade-offs between the wellbeing of individual patients and the total population[155] or the interests of a single hospital and community[156] might make reconciling these two perspectives difficult.

Within-host strategies

For the treatment of individual patients, WHO refers to suboptimum drug concentrations, non-adherence (ie, skipping doses or premature cessation of therapy), and unnecessary treatment as risk factors for emergence of resistance.[157] In theoretical studies, several possible explanations for the dangers of suboptimum therapy have been proposed (table 2), and consensus exists that suboptimum doses facilitate acquisition of resistance in many different pathogens, such as HIV,[72] bacteria,[69,71,77] *Candida albicans*,[73] and *Plasmodium falciparum*.[158,159] Such doses could allow outgrowth of resistant pathogens with costly or inefficient (intermediate) drug-resistance mutations,[66,69–71] which can subsequently evolve to become highly resistant. Even if the drug-resistance mutation is not present at treatment initiation, suboptimum therapy could allow replication of the parental sensitive strain while selecting for resistant mutants (table 2). The same is true for plasmid-borne resistance, since low antimicrobial concentrations often only prevent bacterial replication, and dormant cells can still receive plasmids.[68]

By contrast with the consensus on suboptimum therapy, when to start and stop therapy is less clear. Although the approaches to avoid unnecessary antimicrobial use can lead to late treatment initiation, starting treatment early, and therefore treating when pathogen populations are still small, might prevent emergence and transmission of resistant pathogens.[60,66] However, if the pathogen population declines after an initial peak (eg, as in HIV), treatment could lead to less resistance if it is initiated after that peak.[65] Similarly, for treatment of malaria starting antimalarial treatment after activation of the immune system might be advisable.[160]

Depending on disease, treatment duration can range from several days to lifelong (table 1). Typically, the aim of anti-infective treatment is to eliminate the causative pathogen, which can take years in tuberculosis or might be impossible, as with HIV. When such treatment of a susceptible infection is too short and must be resumed, drug-resistance mutations can accumulate (table 2).[80] Unfortunately, long treatment courses impose strong selection for resistance, because treated patients can only be superinfected, colonised, or re-infected by resistant strains; this suggests a trade-off between elimination of pathogens and creating hosts that are exclusively accessible to resistant strains.[63,84]

For diseases that differ as much as bacterial infections,[80] HIV,[124] and malaria,[98] de-novo acquisition of resistance could be less likely with combination therapy than with single therapy, because pathogens would need several drug-resistance mutations. The clearest downsides of combination therapy are economic costs and clinical side-effects. Additionally, combination can lead to multidrug resistance, which can be even more dangerous than single resistance,[161] because it can either increase the probability of inappropriate initial treatment[162] or, in extreme cases, no other drug might be left to treat the infection. Multidrug resistance can emerge because heterogeneities in drug bioavailability facilitate acquisition of single and subsequently double resistance.[85,106] Also, combination therapy can lead to recombination of plasmid-borne resistance genes on one plasmid, thereby causing transferable multiple resistance.[86] Furthermore, such

therapy might not help prevent resistance if double resistance is as easily acquired as single resistance.[97] In any case, benefits of preventing pathogen acquisition of single resistance and costs of multiresistance need to be weighed against each other carefully.

Between-host strategies

At the between-host level, the additional possibility exists to coordinate treatment for all patients in a given population (eg, a hospital ward or a hospital). Several strategies coordinating the use of different antibiotics have been proposed that aim to increase environmental heterogeneity for the pathogen, thereby inhibiting its spread. One strategy is cycling (ie, scheduled changes of the predominant antibiotic in a hospital ward or hospital). A second is mixing (ie, assignment of consecutive patients to different antibiotics). For endemic pathogens, population dynamic models have predicted that cycling usually performs more poorly than mixing,[61,97] because mixing generates heterogeneity at a finer scale, which is relevant for the ecology of pathogens. By contrast, for an epidemic, such as influenza, generation of a temporally heterogeneous selection pressure might successfully slow down the spread of resistance.[55] Specifically, treatment of the first few cases in the epidemic with a drug A and then switching to a drug B has been shown to strongly reduce the total number of drug-resistant infections.[55] This effect occurs because in an (initially) exponentially growing epidemic, those drug-resistance mutations that emerge in the early phase will cause the largest numbers of infections. In this scenario, these mutations will confer resistance to drug A but not drug B. Hence, the potentially most dangerous mutation events are neutralised for most of the epidemic. In some but not all cases, the epidemic spread of resistance can be limited by prophylactic treatment. For instance, influenza prophylaxis might reduce the incidence of resistance if transmission of the resistant virus is sufficiently low, but increases the incidence if transmission is high.[163] Although various strategies can be implemented during an epidemic, their effectiveness depends strongly on the characteristics of the epidemic.

Conclusions

Rise of drug resistance in pathogens can be divided into two processes: appearance of resistant mutants in a population in which drug-resistance mutations are not present, and the change of their frequency in a population in which resistance mutations are present. Similarly, intervention against resistance can be divided into prevention and management once it has emerged. In individuals, complete prevention of resistance emergence is often a necessary requirement to reduce disease burden. On the epidemiological level, emergence of resistance is unavoidable in many situations and management of resistance is the therefore the primary focus of public-health interventions. For treatment of individuals, the classic recommendation is to "hit hard and early".[164] Early treatment is suggested because in the early phases of infection, the pathogen population is often small and drug-resistance mutations are not easily acquired (table 2). Hard treatment can be achieved by adequate dosing and combination of antimicrobials. Combination therapy is recommended only for HIV, tuberculosis, and malaria, although it might be more generally useful (tables 1–3). However, the guideline of hitting hard and early has a solid theoretical basis only for point-mutations that alter the drug target, the most straightforward way of resistance generation.

There is still a lack of population genetic theory assessing optimum strategies for cases in which resistance is plasmid-borne or phenotypic and not inherited, and when resistance is conferred by molecular mechanisms other than target alteration (table 1). For example, in the case of excreted drug-degrading enzymes, presence of resistant cells leads to decreased susceptibility in all genotypes in a pathogen population. Furthermore, efflux pumps often confer multiple resistance without the need for several mutations. Also, the role of the route of transmission (table 1) and the immune system for resistance emergence is underexplored.

Once resistant strains have emerged within a host, they can spread throughout the population. Interplay between resistance emergence within hosts and spread of resistance in a population is complex. Adequate mapping of this complex interplay with mathematical models might require further development of formal methods that describe both the within-host and between-host levels simultaneously. Additionally, we might be able to learn important lessons from similar biological problems that do not involve infections. An example is cancer and resistance development against anticancer drugs; although the population genetics of resistance development is very similar to that of pathogens, tumour cells are not transmitted from host to host (except for rare cases in animals[165]), and hence all resistance evolves de novo. Comparison of the population genetics of resistance against antimicrobials and anticancer drugs could thus shed light on the role of transmission in resistance development.

For management of resistance once it has emerged in populations of patients, various strategies exist that include the use of several antimicrobials. At the between-host level, a strategy of hitting hard and early with drugs is not predicted to be an optimum strategy, by contrast with theoretical predictions of treatment of individuals. If drug-resistance mutations do not have to emerge, but pre-exist, one management approach is to maximise host heterogeneity. Again, more theoretical work is needed to assess strategies for non-conventional resistance generation. Additionally, the statistical power of clinical studies of treatment strategies should be evaluated; future clinical studies might benefit from collaborations with theoreticians.

Search strategy and selection criteria

Published works were identified with searches of Medline, Google Scholar, Web of Science, and references from relevant articles, without date restrictions; the date of the final search was Oct 16, 2010. Search terms were "drug therapy", "drug resistance", "mutation", "fitness", and "mathematical model". Only papers published in English were included.

Resistance against antimicrobials is a problem that requires population biological and population genetic methods for its solution. The combination of population genetics and epidemiological theory has advanced our understanding of resistance development. Further detailed theoretical studies that incorporate more realistic resistance-generating mechanisms into models of population genetics are needed. Insights obtained from these models and the assumptions on which they are based should be experimentally and clinically assessed. The future of this subject lies in the development of a general framework for the evolution of resistance that allows comparison of results obtained from studies of different pathogens.

Contributors

All authors searched for and read published work and contributed to the writing of the Review.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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