

Micro-evolution and emergence of pathogens

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Abstract

Changes in the epidemiology of infectious diseases are the direct result of ecological and evolutionary changes in hosts and parasites. Precisely what the causal processes are is rarely known in any particular case, and this hinders the design of appropriate control strategies. This is particularly so for emerging infections, as opportunity is rapidly lost to study the ecological parameters which might have affected initial emergence. However, molecular evolutionary studies of the pathogens can yield data which discriminate between possible causes. The current distribution of DNA sequence variation is important information which may reveal past and current changes in pathogen population structures, and can also identify adaptive changes in pathogen genes which have affected their evolution. Such studies have been quite intensively performed on particular viral and bacterial pathogens, and some of the successes of these are noted here. Approaches to understanding the recent evolution of eukaryotic pathogens are outlined, with particular reference to current problems of emerging zoonoses, and changes in virulence and drug resistance. © 2000 Australian Society for Parasitology Inc. Published by Elsevier Science Ltd. All rights reserved.

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1. Introduction

Molecular evolutionary studies have contributed to understanding the epidemiological emergence of pathogens. This is best illustrated by studies on human immunodeficiency viruses (HIV), at global [1–3], local [4,5], and within-host [6] levels. More broadly, emerging pathogens consist of a ‘mixed bag’ of viruses, bacteria, fungi, protozoa, and metazoa, and the causes of their epidemiological emergence vary greatly. Research questions and methods which have been fruitful in one case may not be relevant in others, and the potential role of molecular evolutionary analyses requires some general consideration.

Deciding what constitutes an ‘emerging disease’ is somewhat arbitrary, although a working definition given in the context of public health in the USA is a disease whose incidence in humans has increased within the last 2 decades or threatens to increase in the near future [7]. For discussion of biological principles, a more broad consideration is preferable, as all infectious diseases have ‘emerged’ at one time or another. There are data to indicate that some endemic human diseases of historical antiquity, such as malaria and tuberculosis, have actually only emerged to

be common in human populations in the relatively recent past [8–10]. Also, currently emerging diseases of diverse animal species are of importance in their own right, and may contribute to a general understanding [11,12].

Molecular genetic ‘typing’ of pathogen isolates is now widely practised. The genetic characters are essentially DNA sequences, although it is sometimes adequately informative and convenient to type defined sets of marker loci, in the form of single nucleotide polymorphisms (SNPs), simple sequence repeat microsatellite polymorphisms, or polymorphic gene products which are electrophoretically or serologically distinguished. The loci, and the purposes of their study, fall into two main classes: (i) gene loci with putatively functional alleles, which may determine phenotypes such as host-specificity, virulence, or drug-resistance; (ii) loci with neutral alleles, which can give an indication of the way in which genetic variation has been affected by past and current population structure.

At the outset of an investigation, assumptions need to be stated about whether alleles at particular loci are functionally important and thus under selection, or whether they are neutral. If it is aimed to actually identify which loci have functional alleles under selection, particular design and analysis is required which may differ for studies on pathogens which have undergone host-switching [13], or which are epidemic [14] or endemic [15]. Here we discuss some of

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the effective molecular approaches to understanding the evolution of emerging pathogens. Some of the examples are of studies on viruses and bacteria, which may help us to consider the potential of new approaches to studying eukaryotic pathogens.

2. The zoonotic–anthroponotic spectrum

We first consider emerging infections with respect to their zoonotic status, as this helps to determine which types of epidemiological issues need investigation. Table 1 presents a scheme which groups human infections into five major categories (A–E), ranging from exclusively zoonotic (Category A) through to non-zoonotic, i.e. exclusively anthroponotic (Category E).

For exclusively zoonotic infections (Category A), known risk factors are usually associated with exposure to infected animal tissue, body fluids, or excreta, or exposure to vectors which have fed the on host animal species. It is unlikely that adaptive genetic changes in the pathogens have occurred in human infections, and if such changes did occur they have not affected the epidemiology as humans are ‘dead-end’ hosts. There may be genetic heterogeneity among morphologically indistinguishable pathogens in different animal hosts, with an unknown subset causing human infections. In such a case, descriptive molecular genotypic characterisation of putatively zoonotic pathogens can help identify likely reservoir host species, namely those infected with pathogens having similar genotypes to the ones infecting humans [16].

Zoonotic infections which are occasionally transmitted from one person to another (Category B) allow more scope for evolutionary study. Geographical or temporal clusters of cases in humans may be caused either by a focus of high risk of contact with reservoir hosts, or by outbreaks involving human-human transmission. Resolving whether human-human transmission has occurred involves

a combination of classical epidemiology, and molecular genetic typing of pathogens (to determine whether a particular pathogen clone or lineage has expanded to become the predominant one in human cases). The data may discriminate genotypes in a simple qualitative manner, or phylogenetic analyses may be applied to estimate the relationship among genotypes, but a simple epidemiological conclusion is aimed for in either case. If results indicate that clusters of cases are caused by human-human transmission, this may raise a hypothesis of adaptive changes in the pathogen, which would subsequently be a matter for separate study. The means of testing this are (i) laboratory experiments on isolated clones to test whether there is a systematic difference in infectivity or tropism in available in vivo or in vitro models, and/or (ii) molecular evolutionary analysis to test for evidence of positive selection on the sequence of genes which are candidates for determining host-specificity. Adaptive changes in pathogens may also allow further establishment of transmission in human populations, so this is discussed more broadly later.

Pathogens which are zoonotically transmitted but which also may be maintained within human populations (Category C), present uncertainties for epidemiology and control. The relative importance of zoonotic transmission may not be clearly known, and may vary among human populations. Pathogens with differing host affiliations may be phenotypically similar, but if there is reproductive isolation between them they may be genotypically distinct. Illustrative examples are provided by studies of intestinal nematode infections [17], including human and pig infections with *Ascaris* roundworms (*Ascaris lumbricoides* and *Ascaris suum*, closely-related and virtually indistinguishable by morphology), using nuclear and mitochondrial DNA haplotype analysis. In the USA, human infections with *Ascaris* are rare, and contact with pigs is a factor associated with infection. Worms sampled from these sporadic infections are genetically similar to *A. suum* in American pigs and differ-

Table 1

A scheme of infectious pathogens of humans categorised with respect to their place on the zoonotic–anthroponotic spectrum

| Category | Definition | Examples |
|-------------------------|--|--|
| A. Exclusively zoonotic | All human infections are acquired from animals, or by animal-vector-human transmission. No transmission between human ‘dead-end’ hosts | Hantaviruses, Rabies virus, <i>Borrelia burgdorferi</i> , <i>Toxocara canis</i> |
| B. Mainly zoonotic | Animal reservoirs are required to maintain the pathogen, and account for most human infections. Transmission between humans can occasionally occur, and may contribute to local outbreaks or epidemics | Ebola virus, <i>E. coli</i> O157, <i>Mycobacterium bovis</i> , <i>Trypanosoma brucei</i> |
| C. Partially zoonotic | Some human infections are acquired from animals, but infection is also endemic in humans. Prevention of zoonotic infection may not eradicate endemic infection, or vice versa | <i>Giardia intestinalis</i> , <i>Cryptosporidium parvum</i> , <i>Brugia malayi</i> |
| D. Originally zoonotic | An original animal host is known or inferred, but infection is now maintained mainly or exclusively in human host populations | HIV-1, HIV-2, HTLV-1 |
| E. Not zoonotic | All human infections are acquired from other humans. No animal reservoir is known now or at any time in history | Hepatitis B virus, <i>Mycobacterium tuberculosis</i> , <i>Plasmodium falciparum</i> , <i>Onchocerca volvulus</i> |

ent from *A. lumbricoides* in endemic human populations, confirming the hypothesis of zoonotic transmission [18]. On the other hand, in Guatemalan villages, humans and pigs are sympatric but the two *Ascaris* species are separated in their respective human and pig host populations, so worms from human hosts have *A. lumbricoides* genotypes and zoonotic transmission has not been observed [19]. There is a dilution effect on sampling in Guatemala, where *A. lumbricoides* is so highly endemic that rare zoonotic infections by *A. suum* will only account for a minute fraction of worms in humans and thus they are unlikely to be reported.

There are other anthroponotic parasites which can also, to an unknown extent, be zoonotically transmitted. For example, the globally distributed malaria parasite *Plasmodium malariae* occurs in the Amazonian region and the Guianas of South America, where it is also enzootic (under the synonym *Plasmodium brasilianum*) in several rainforest monkey species [20]. Its distribution in Amazonia is quite focal, and it is apparently re-emerging in the Guianas, although the causes for this are unknown. The filarial nematode *Brugia malayi* in endemic rural areas of Southeast Asia is also enzootic in mammals such as silvered leaf monkeys. There is heterogeneity in microfilarial periodicity of different *B. malayi* strains, which apparently does not reliably indicate host affiliation [21]. For both *P. malariae* and *B. malayi*, there are available DNA sequences of mitochondrial and nuclear loci [22,23], and some sequence polymorphisms are already identified in nuclear loci [20,24,25]. Study of these loci might reveal population genetic structures which would allow a discrimination of zoonotic and anthroponotic populations, so that zoonotic transmission (which may be emerging or declining) may be quantified.

The problem of ‘dilution’ by phenotypically similar endemic infections, mentioned above, makes it difficult to detect emerging zoonoses by routine surveillance. This is of most concern with respect to infections which can rapidly emerge from animal to human hosts, and are now predominantly endemic in humans (Category D). The zoonotic phase may be missed entirely by investigators and only traced retrospectively, as has been the case for zoonotic origins of HIV-1 from chimpanzees [1,2] and HIV-2 from sooty mangabeys [2,26]. Due to the overwhelming burden of cases of infection with the established types of HIV-1 and HIV-2 in Africa, it is likely that existing surveillance will not quickly detect new (and possibly even more virulent) types currently being zoonotically transmitted from African primates in the bush meat trade [2].

Understanding the past emergence of human pathogens with more remote or uncertain zoonotic origins is of academic interest, and may also be of predictive epidemiological importance. Human T cell lympho trophic virus-1 (HTLV-1) infection is endemic in several widely-separated human populations, but the origins of these endemic foci are obscure. If HTLV-1 is an ancient anthroponotic virus, it raises the question of why there is not a more continuous endemic distribution. Comparison of HTLV-1 DNA

sequences with those of related simian viruses (STLV-1) in African and Asian monkeys indicates that HTLV-1 probably has several zoonotic origins in Africa (where original hosts include mandrills) and at least one in Asia [27]. Thus, there may also be a possibility of future zoonotic emergence of new STLV-1/HTLV-1 lineages, particularly from hunting and consumption of primates. On the other hand, Hepatitis B viruses (HBV) in humans belong to diverse lineages which do not clearly cluster with any of the particular types of HBV-like viruses isolated from other primate species (including chimpanzees, gibbons, orang-utans, and woolly monkeys) [28]. Since no putative zoonotic origin of HBV has been revealed, the likelihood of future emergence of new HBV lineages is unknown.

Human pathogens which have no known or inferred original animal host, are here considered as non-zoonotic (Category E). As for HBV, the origins of these pathogens remain obscure. However, DNA sequences indicate that modern *Mycobacterium tuberculosis* and *Plasmodium falciparum* only emerged as common human pathogens within the last few tens of thousands of years [8–10]. A clearer understanding of the emergence and evolution of these currently-endemic species will contribute to studies of host-parasite evolution. Tuberculosis and malaria may have had major selective effects on alleles of human immune response genes [29], and it is of interest to immunogenetics if we can define when such selection could have occurred. Molecular population genetic analysis of haemoglobin variants associated with protection from malaria is consistent with a hypothesis that selection on these occurred mainly within the last few thousands (or tens of thousands) of years [30].

3. Processes of emergence in new host populations

An important question is how a pathogen changes its position on the zoonotic-anthroponotic spectrum (Fig. 1). For example, what factors allow an exclusively zoonotic pathogen to become occasionally transmitted from one human to another, (i.e. to move from Category A to B)? Then, how does transmission of some pathogen species (or intra-specific lineages) become more predominantly anthroponotic (Categories C and D)? These processes are symbolically represented by arrows in Fig. 1. If the causes of these processes can be understood for previous and current emerging infectious diseases, factors affecting the likelihood of future pathogen emergence might be predicted. Such prediction might allow targeted modification of emergence risks.

The causes are primarily (i) pathogen adaptation to hosts, and (ii) extrinsic ecological changes in host populations. Retrospective molecular evolutionary analyses, and experiments on tropism of pathogen isolates, can help to distinguish the effects of each. At least three HIV-1 lineages have emerged independently in human hosts from Simian immunodeficiency virus-1 (SIV-1) ancestors in chimpanzee

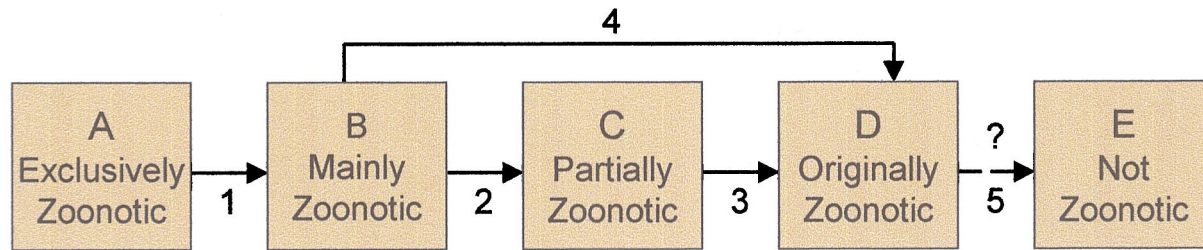


Fig. 1. Scheme representing the changing epidemiological status of pathogens along the zoonotic–anthropothonic spectrum. The boxed categories are as defined in Table 1. The arrows represent changes in transmission (in an anthropothonic direction) which occur due to different causes, some of which are discussed with examples in the text. Evolutionary and ecological changes underlie the movement between categories A–D (arrows 1–4). It is possible that some pathogens which are not known to have been zoonotic (Category E) may once have been so (Category D), and thus the broken arrow 5 represents a loss of traceable evidence rather than an actual epidemiological change.

hosts [1,2]. These lineages are phylogenetically disparate, but the most common one in humans (HIV-1 group M) exhibits strong host-specificity. The molecular basis of host-specificity of SIV-1/HIV-1 viruses is a subject of much laboratory investigation [31], and there is an apparent correlation with the peptide sequence of the central part of the V3 loop in the envelope protein [1,2]. This putatively adaptive sequence evolution has not occurred in the rarest of the types in humans (HIV-1 group N) which has only been isolated in a region of Cameroon where sympatric SIV-1cpz in chimpanzees have virtually identical sequences. A test of this particular adaptive hypothesis will thus be possible if the HIV-1 group N emerges epidemically, the expectation being that the central part of the V3 loop sequence would undergo convergent evolution and become similar to that in the pandemic group M.

Rabies transmission is exclusively zoonotic, but changes in the reservoir host populations have affected the epidemiology in humans. The virus is transmitted by various carnivores, although dogs are the major hosts in many regions [32], and the evolutionary processes determining emergence of rabies in new hosts are unknown. Red foxes became the primary reservoir hosts in Europe only during the last century, prior to which dogs and wolves were major hosts. The known origin of rabies into foxes in eastern Europe and subsequent spread westwards, together with infection of an introduced exotic susceptible host species (raccoon dogs), has allowed a molecular evolutionary study of host-specificity with a search for candidate adaptive changes [13]. The DNA sequences reveal a geographical and host-structured viral phylogeny consistent with recent spread through the red fox population from East to West Europe, and a single introduction into raccoon dogs from North-Eastern European foxes. Statistical tests indicated that a few codon changes might not be selectively neutral, and one of these (codon 101 of the nucleoprotein gene) is associated with the lineage introduced into red foxes, so further work could test whether this is adaptive [13].

Such phylogenetic analyses of emerging lineages may not be similarly applied to all pathogen species. The nucleotide substitution rate of viruses such as HIV-1 [33], rabies [13],

and flaviviruses [34] exceeds that of bacteria and eukaryotes by several orders of magnitude. Thus, phylogenetic trees of the latter do not so accurately trace very new lineages, as relatively few nucleotide substitutions have occurred recently. Table 2 presents DNA sequence-based estimated emergence times of some important human pathogens, and it may be noted that these are shorter and more precise for the viral examples. The lineages which can be resolved in bacterial and eukaryote species are much older than those of viruses, and most existing sequence polymorphism is ancient (unless species have recently emerged from only one or a few founder clones, and thus lost ancestral polymorphism). Recent evolution of bacteria depends more substantially on horizontal transfer and recombination of genes between bacterial clones or species, so that recent lineages contain mosaic elements of other older lineages [35].

Diploid eukaryote populations are particularly likely to retain ancient polymorphisms, as a single heterozygote individual may contain a substantial proportion of the population's nucleotide diversity, so even if a new population is founded by only one or a few individuals the loss of polymorphism may not be complete. Balancing selection can also maintain very ancient alleles, and this may explain highly divergent alleles of some merozoite proteins of *P. falciparum* which have high identity with the homologue in the chimpanzee parasite *Plasmodium reichenowi* [36,37]. Such ancient alleles are unlikely to be maintained as discrete haplotypes, as recombination (whether mediated

Table 2
Estimated times since emergence of some major human pathogens, based on DNA sequence analysis

| Pathogen | Estimated number of years since first major emergence | Ref. |
|-----------------------------------|---|-------|
| HIV-1 | ~70 | [3] |
| Japanese encephalitis virus | ~130 | [34] |
| <i>Mycobacterium tuberculosis</i> | <20 000 | [10] |
| <i>Plasmodium falciparum</i> | <50 000 | [8,9] |

by sexuality or plasmids) shuffles the allelic sequences. This occurs by sexual reproduction in *P. falciparum* [38,39] which is in no way compromised by its evolutionarily recent epidemiological emergence.

In the case of some emerging pathogens in which reproduction is completely asexual and there are no horizontally mobile genetic elements, recombination will have little effect [40]. Independent of recombination, the genomic organisation of coding loci influences the evolution and expression of new phenotypes associated with emergence. The existence of hypervariable homopolymeric nucleotide loci is likely to allow phase switching of expression of virulence genes in bacteria such as *Campylobacter jejuni* [41] and may be a functional aspect of some of the abundant microsatellite loci in eukaryotes such as *P. falciparum* [39]. With a variety of possible mechanisms, the potentially rapid evolution of virulence and drug resistance warrants some discussion here.

4. The evolution of virulence

Natural selection favours the most transmissible genotypes because they pass more offspring to the next generation. Where elevated transmission is harmful to the host, the associated costs to the pathogen may act as a brake on the evolution of ever more transmissible and ever more virulent microbes.

Broad consideration [42] and analysis [43] of the evolution of human infectious diseases has helped to clarify some general principles about virulence and transmission of pathogens. These are appealing as they apply to some extent regardless of the precise molecular basis of virulence. For example, it is predicted that effective sanitation programs will ultimately lead to the evolution of more benign water-borne or vector-borne pathogens because reduced efficiency of transmission requires more extended association with the host.

These predictions may be applied even when the genetic basis of virulence, (e.g. a single locus gene, a multi-gene family, or a co-adapted pathogenicity island) or the mechanisms of structural change, (e.g. point mutation, insertion/deletion at homopolymeric or simple repeat loci, plasmid integration, meiotic recombination) is not known. However, central to these predictions is the assumption that transmission and virulence are functionally associated. Studies of *Plasmodium chabaudi* malaria in mice have established that virulence and transmission success are genetically correlated [44], and it seems likely that some of the observed variation in the severity of *P. falciparum* malaria in humans may be due to virulence polymorphisms. It has recently been observed that parasites isolated from severe malaria patients in Thailand have a significantly higher replication rate in vitro than parasites from mild malaria patients [45]. The adaptive significance of such virulence needs investigation, as the transmission forms of malaria

parasites are specialised sexual gametocytes, distinct from the replicative asexual forms which cause disease.

Within-host competition may select for rapid multiplication which is of short term competitive advantage. In *P. chabaudi*, virulence and transmission success were both increased in infections with two clones of the parasite compared with uniclonal infections [46]. This result was not explainable by effects on parasite density so the role of competition between clones could not be confirmed. The theoretical probability that virulence and transmissibility of *P. falciparum* will evolve to be greater in populations where co-infections with two or more clones are common (due to within-host competition between parasites) has not yet been tested by empirical data. It has been known for some time that the average number of clones per infection does not differ between sympatric groups of patients with severe and mild malaria [47]. However, if virulence of a parasite is a genetically fixed property (rather than being facultatively expressed in response to the presence of other clones in a given infection), comparisons between different endemic areas may be more relevant than comparisons between infections within the same area. Careful control for confounding variables will be important in such ecological comparisons.

5. The emergence of drug resistant pathogens

'Bacteria, viruses, fungi, and protozoa resistant to previously effective chemotherapeutic agents may well be the most significant single source of emerging and re-emerging pathogens in the developed and overdeveloped world and the most important infectious threat to the future of human health' (Levin) [48].

While the evolution of drug resistance is generally accepted to be inevitable, there have historically been wide discrepancies in the rate of evolution of resistance. This applies both to the term of effectiveness of different drugs against the same pathogen and of the same drug against different pathogens. Using molecular genetic approaches progress has been made in the understanding of mechanisms of resistance and their evolutionary origins. From this, it is hoped that we can learn from past experience and identify those factors which hasten the rate of evolution of resistance, opening the way for new approaches to anticipatory management of resistance which will maximise the term of effectiveness of new drugs.

There are diverse mechanisms underlying resistance [49], one of the most common being adaptive modification of the drug target. A well studied example of this is resistance to antifolate drugs in malaria parasites. These drugs inhibit parasite folate metabolism by binding with the active site of key enzymes. Adaptive modification of the active site of dihydrofolate reductase (DHFR, the target of, e.g. pyrimethamine) and dihydropteroate synthase (DHPS, the target of, e.g. sulphadoxine) reduces the affinity of the drug for its

target. The structural adaptations take the form of single point mutations coding for amino acid changes which alter the conformation of the drug binding pocket. In popu-

lations where resistance is longstanding, e.g. in Southeast Asia, a number of different point mutations have accumulated (Fig. 2). In elegant culture experiments, mutant *dhps* alleles with different point mutation haplotypes were transfected into sensitive parasites, showing that individual amino acid substitutions confer increasing degrees of resistance in the parasite [50]. For example, the substitution of alanine with glycine at position 437 conferred a five-fold increase in sulphadoxine resistance, and addition of further changes at positions 436 and 613 increased the level of resistance to 24-fold over the sensitive type.

There is generally an inverse relationship between the extent to which a drug is used and the time before resistance emerges. For example, mass drug administration (MDA) programs in the malaria eradication era in the 1950s are believed to have generated the first foci from which chloroquine resistance spread world-wide [51,52]. In some cases the drug was added to salt so that people ingested varying doses in their daily diet. Such programs deliver a low and fluctuating dosage which facilitates the incremental evolution of resistance as subtherapeutic levels of drug allow the cumulative acquisition of genetic changes of small effect [53]. Certain pharmacokinetic properties of a drug can also predispose toward emergence of resistance. If they are irregularly absorbed from the gut or have a long elimination half life, subcurative doses are present for extended periods of time. A major cause for concern is the continuing practice of antibiotic use in agriculture, particularly the administration of low doses as growth enhancers in live-stock [54].

Reports of immediate emergence of resistance following wide scale MDA of pyrimethamine both in dietary salt [52] and in curative doses [55], indicates that resistance alleles were pre-existent or that they arise rapidly by mutation. The different arrangements of point substitutions in *dhfr* genes shown in Fig. 2 illustrate how the same resistance mechanism has evolved independently in different *P. falciparum* populations. With the benefit of hindsight, we would now conclude that de novo emergence of resistance to pyrimethamine is predictable. Resistance to chloroquine appears to have been rather less so. After chloroquinised salt was administered in projects worldwide, it was at least 3 years before the emergence of resistance in Southeast Asian and South American sites [52]. It is understood that chloroquine resistance is polygenic and two resistance loci have so far been identified *pfmdr 1* on chromosome 5 [56] and a gene in the region of *cg2* on chromosome 7 [57]. It has been claimed that as few as two or three mutation events may account for all chloroquine resistant *P. falciparum* [51].

Mathematical models predict that resistance with a single genetic mechanism will emerge much more rapidly than resistance requiring multiple genetic mechanisms [58,59], and data show that antibiotics with multiple targets have a longer effective lifespan [60]. Drugs with multiple targets can be made artificially by combining drugs which have different modes of action and this is why, for example, pyri-

A. DHFR

| Position Sensitive Genotype Geographic location | 16 Ala | 51 Asn | 59 Cys | 108 Ser | 164 Ile |
|---|-----------|-----------|-----------|------------|------------|
| Kenya [64] | . | . | . | Asn | . |
| | . | Ile | . | Asn | . |
| | . | . | Arg | Asn | . |
| | . | Ile | Arg | Asn | . |
| Tanzania [65] | . | . | . | Asn | . |
| | . | Ile | . | Asn | . |
| | . | . | Arg | Asn | . |
| | . | Ile | Arg | Asn | . |
| Mali [65] | . | Ile | Arg | Asn | . |
| Vietnam [65] | . | Ile | Arg | Asn | . |
| | . | . | Arg | Asn | Leu |
| | . | Ile | Arg | Asn | Leu |
| Middle East [65] | . | . | . | Asn | . |
| | . | . | Arg | Asn | . |
| | . | Ile | . | Asn | . |
| | Ser | . | Arg | . | . |
| Peru [66] | . | . | . | Asn | . |
| | . | . | . | Asn | Leu |
| | . | Ile | . | Asn | Leu |

B. DHPS

| Position Sensitive Genotype Geographic location | 436 Ser | 437 Ala | 540 Lys | 581 Ala | 613 Ala |
|---|------------|------------|------------|------------|------------|
| Kenya [64] | . | Gly | . | . | . |
| | Ala | Gly | . | . | . |
| | . | Gly | Glu | . | . |
| | Ala | . | . | . | . |
| East Africa [65] | . | . | Glu | . | . |
| | . | Gly | . | Gly | . |
| | . | Gly | Glu | Gly | . |
| | . | Gly | Glu | . | . |
| | Ala | . | . | . | . |
| Mali [65] | . | Gly | . | Gly | . |
| | . | Gly | . | . | . |
| | Ala | Gly | . | Gly | . |
| SE Asia [65] | . | Gly | . | Gly | . |
| | Ala | Gly | Glu | . | . |
| | Ala | Gly | Glu | Gly | . |
| | . | Gly | Glu | Gly | . |
| | Ala | Gly | . | . | . |
| | Phe | Gly | . | . | Ser |
| Phe | Gly | . | . | Thr | |
| Peru [66] | . | Gly | Glu | Gly | . |

Fig. 2. Geographical occurrence of different drug resistance alleles in *P. falciparum* enzymes: (A) dihydrofolate reductase, DHFR; (B) dihydropteroate synthase, DHPS. Amino acid substitutions in resistance alleles are shown compared with amino acids in the allele sensitive to pyrimethamine (A) and sulphadoxine (B). Refs. [64–66] are given for the data from each geographical location. Positions which are identical to the sensitive type are shown with a dot (-).

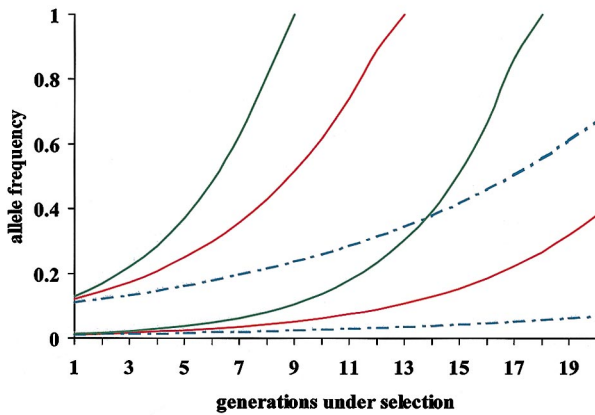


Fig. 3. Changes in the frequency of alleles which confer different degrees of resistance a constant level of drug pressure. Three resistance alleles, with selection coefficients of 0.1 (blue dashed), 0.2 (red) and 0.3 (green) compared with sensitive types. Changes starting from initial frequencies of 0.1 and 0.01 are shown over 20 generations (i.e. 20 complete parasite life cycles).

methamine is combined with sulphadoxine in a single tablet, Fansidar™ (Roche). Recently much interest has focused upon the use of drugs in combination with artesunate as a means of proactively delaying the emergence of resistance [61].

Resistance distribution reflects the geographic patterns of drug use, and the rate of spread of resistance is subject to strong selective forces. A genetics transmission model which examined the forces determining the rate of spread of drug resistance in malaria showed that the rate of change of frequency of resistance genetic variants is a function of drug coverage and parasite transmission rates [59]. Fig. 3 illustrates the likely rate of spread of different resistance alleles (with different selection coefficients) under equal drug pressure. A resistance allele with a selection coefficient of 0.3 (30% greater fitness on average than the sensitive type) spreads much more rapidly than an allele with a selection coefficient of 0.1, so that a tenfold lower starting allele frequency (0.01 vs. 0.1, Fig. 3) is quickly made up for (note the lines crossing after only 14 generations in Fig. 3).

Resistance alleles may confer fitness costs, so that in the absence of drugs the resistant pathogen has a selective disadvantage. A mathematical model of antibiotic resistance [62] showed that modest costs of fitness can make a large difference to the equilibrium frequency of resistant forms, depending also on the intensity of drug selection [48]. Unfortunately the coevolution of secondary adaptations which compensate for the costs of antibiotic resistance have shown to exist [63]. Hence, it may be over optimistic to hope that temporary cessation of drug use will cause selection for drug sensitivity in pathogen populations. Future research will need to focus on how to limit the rate of emergence of novel resistance alleles, as well as how to develop new drugs with novel modes of action. The identification of many new potential drug targets encoded in

pathogen genomes encourages the prospects for the latter, but genetic and economic realities may favour the former.

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