Drug Resistance: Mechanisms and Management

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The Future of Infectious Disease

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The influenza pandemic of 1918-1919 was a disease outbreak of historic dimensions. In the U.S., it was a sharp punctuation of the great improvement of life expectancy between 1900-1950 (figure 1). Throughout the world it accounted for 20 to 25 million excess deaths in one year, formidable even against the backdrop of other plagues and of ongoing parasitic and diarrhoeal disease.

The infectious agent was not available for study at that time. We have little doubt that the outbreak was a manifestation of the incessant evolution of the influenza virus.[1] However, very recently the U.S. Armed Forces Institute of Pathology recovered with PCR technology genetic fragments of the 1918 influenza virus.[2] Less than 10% of the entire genome has been recovered to date, but recovery of complete sequences is likely. Although the target genes have not yet provided a clue as to why the 1918 influenza was so devastating, they demonstrate the enormous potential of today's molecular biology tools.

These tools will enable us to better study paleovirology and paleomicrobiology. We are accustomed to stereotyping historical disease outbreaks as if we really knew what they were, but we really know very little detail about their genetic features. For example, we talk about the great historic plagues as if they indeed were Yersinia or cholera or malaria. We should look forward to finding out about the 14th century black death, if it was indeed *Yersinia pestis*. Although clinically unmistakable, that is not to say it was caused by the identical genotype of present Yersinia strains.

We need to look ahead as well as back. In this century, emerging and re-emerging infections have stimulated flurries of interest, but in general the populations of economically advanced countries have been complacent about infectious diseases ever since the introduction of antibiotics. The effect of antibiotics on acute infections and tuberculosis as well as the effect of polio vaccination led to a national, almost worldwide, redirection of attention to chronic and constitutional diseases. However, the HIV pandemic in the early 1980s caught us off guard, reminding us that there are many more infectious agents in the world. It is fortuitous that retroviruses had already been studied from the perspective of cancer aetiology; otherwise, we would have had no scientific platform whatsoever for coping with HIV and AIDS. Beyond AIDS, the challenges of emerging and re-emerging infections are legion (tables 1, 2).

Globally, we are engaged in a type of race, enmeshing our ecologic circumstances with evolutionary changes in our predatory competitors. To our advantage, we have wonderful new technology; we have rising life expectancy curves. To our disadvantage, we have crowding; we have social, political, economic, and hygienic stratification. We

have crowded together a hotbed of opportunity for infectious agents to spread over a significant part of the population. Affluent and mobile people are ready, willing, and able to carry afflictions all over the world within 24 hours' notice. This condensation, stratification, and mobility are unique, defining us as a very different species from what we were 100 years ago. We are enabled by a different set of technologies. But despite many potential defences — vaccines, antibiotics, diagnostic tools — we are intrinsically more vulnerable than before, at least in terms of pandemic and communicable diseases.

We could imaginably adapt in a Darwinian fashion, but the odds are stacked against us. We cannot compete with microorganisms whose populations are measured in exponents of 10^12, 10^14, 10^16 over periods of days. Darwinian natural selection has led to the evolution of our species but at a terrible cost. If we were to rely strictly on biologic selection to respond to the selective factors of infectious disease, the population would fluctuate from billions down to perhaps millions before slowly rising again.

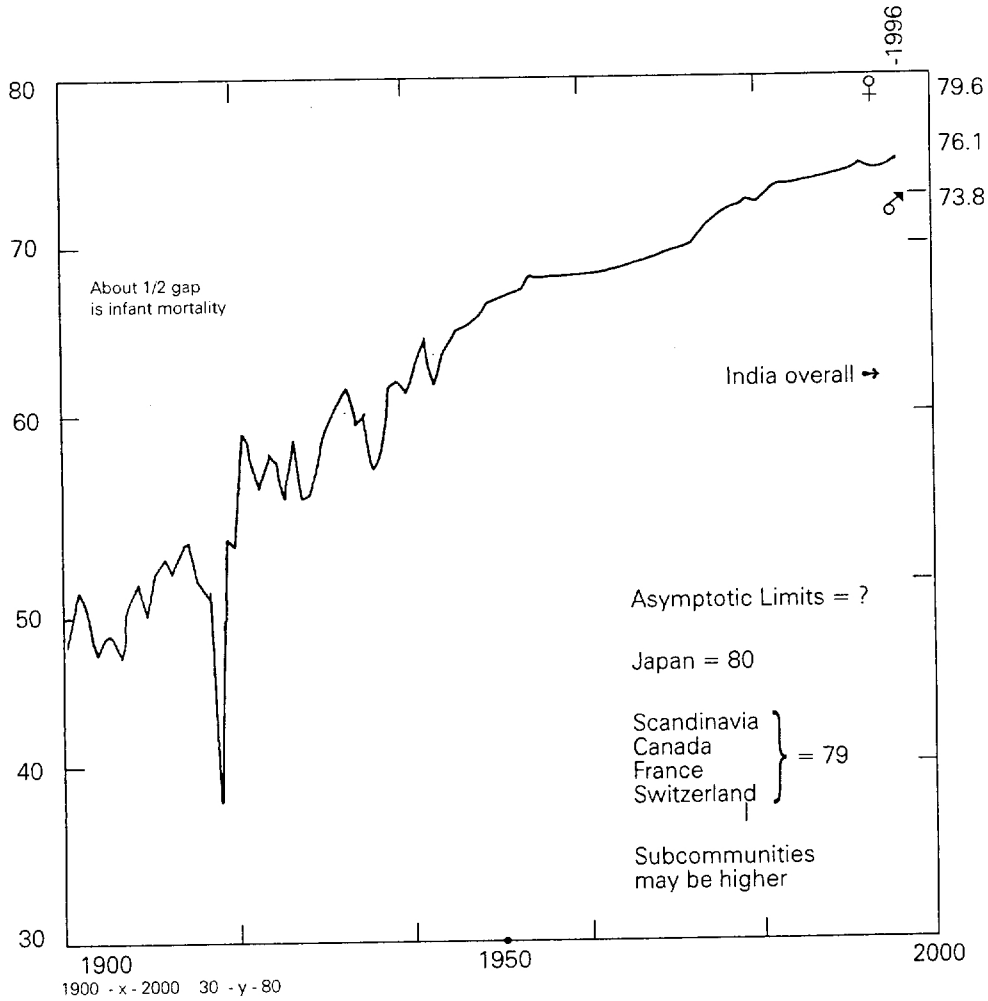


Figure 1. U.S. life expectancy at birth. Twentieth Century.

Table 1. Examples of pathogenic microbes and infectious diseases recognised since 1973*

Year	Microbe	Type	Disease
1973	Rotavirus	Virus	Major cause of infantile diarrhoea worldwide
1975	Parvovirus B19	Virus	Aplastic crisis in chronic haemolytic anaemia
1976	*Cryptosporidium parvum*	Parasite	Acute and chronic diarrhoea
1977	Ebola virus	Virus	Ebola haemorrhagic fever
1977	*Legionella pneumophila*	Bacteria	Legionnaires' disease
1977	Hantaan virus	Virus	Haemorrhagic fever with renal syndrome (HRFS)
1977	*Campylobacter jejuni*	Bacteria	Enteric pathogens distributed globally
1980	Human T-lymphotropic virus I (HTLV-I)	Virus	T-cell lymphoma-leukaemia
1981	Toxic producing strains of *Staphylococus aureus*	Bacteria	Toxic shock syndrome (tampon use)
1982	*Escherichia coli O157:H7*	Bacteria	Haemorrhagic colitis; haemolytic uraemic syndrome
1982	HTLV-II	Virus	Hairy cell leukaemia
1982	*Borrelia burgdorferi*	Bacteria	Lyme disease
1983	Human immunodeficiency virus (HIV)	Virus	Acquired immunodeficiency syndrome (AIDS)
1983	*Helicobacter pylori*	Bacteria	Peptic ulcer disease
1985	*Enterocytozoon bieneusi*	Parasite	Persistent diarrhoea
1986	*Cyclospora cayatanensis*	Parasite	Persistent diarrhoea
1988	Human herpesvirus-6 (HHV-6)	Virus	Roseola subitum
1988	Hepatitis E	Virus	Enterically transmitted non-A, non-B hepatitis
1989	*Ehrlichia chafeensis*	Bacteria	Human ehrlichiosis
1989	Hepatitis C	Virus	Parenterally transmitted non-A, non-B liver infection
1991	Guanarito virus	Virus	Venezuelan haemorrhagic fever
1991	*Encephalitozoon hellem*	Parasite	Conjunctivitis, disseminated disease
1991	New species of *Babesia*	Parasite	Atypical babesiosis
1992	*Vibrio cholerae O139*	Bacteria	New strain associated with epidemic cholera
1992	*Bartonella henselae*	Bacteria	Cat-scratch disease; bacillary angiomatosis
1993	Sin nombre virus	Virus	Adult respiratory distress syndrome
1993	*Encephalitozoon cuniculi*	Parasite	Disseminated disease
1994	Sabia virus	Virus	Brazilian haemorrhagic fever
1995	HHV-8	Virus	Associated with Kaposi sarcoma in AIDS patients

** Adapted from (6)*

Table 2. Re-emerging infections during the last 2 decades and factors contributing to their re-emergence*

Disease or Agent	Factors in Re-emergence
Viral	
Rabies	Breakdown in public health measures; changes in land use; travel
Dengue/dengue haemorrhagic fever	Transportation, travel and migration; urbanisation
Yellow Fever	Favourable conditions for mosquito vector
Parasitic	
Malaria	Drug and insecticide resistance; civil strife; lack of economic resources
Schistosomiasis	Dam construction, improved irrigation, and ecological changes favouring the snail host
Neurocysticercosis	Immigration
Acanthamoebiasis	Introduction of soft contact lenses
Visceral leishmaniasis	War, population displacement, immigration, habitat changes favourable to the insect vector, an increase in immunocompromised human hosts
Toxoplasmosis	Increase in immunocompromised human hosts
Giardiasis	Increased use of child-care facilities
Echinococcosis	Ecological changes that affect the habitats of the intermediate (animal) hosts
Bacterial	
Group A Streptococcus	Uncertain
Trench fever	Breakdown of public health measures
Plague	Economic development: land use
Diphtheria	Interruption of immunisation programme due to political changes
Tuberculosis	Human demographics and behaviour; industry and technology; international commerce and travel; breakdown of public health measures; microbial adaptation
Pertussis	Refusal to vaccinate in some parts of the world because of the belief that injections or vaccines are not safe
Salmonella	Industry and technology; human demographics and behaviour; microbial adaptation; food changes
Pneumococcus	Human demographics; microbial adaptation; international travel and commerce; misuse and overuse of antibiotics
Cholera	Travel: a new strain (O139) apparently introduced to South America from Asia by ship, with spread facilitated by reduced water chlorination and also food

** Adapted from (6)*

Table 3 . Genetic Evolution

Microbes (bacteria, viruses, fungi, protozoa):
Rapid and incessant
Huge population sizes 10^14+ and generation times in minutes vs years

Intraclonal:
DNA replication — may be error-prone — in sea of mutagens
sunlight; unshielded chemicals, incl. natural products
RNA replication — intrinsically unedited, > 10^-3.
swarm species
haploid: immediate manifestation; but partial recessives not accumulated
contra multicopy plasmids
amplification
site-directed inversions and transpositions: phase variation
?? Other specifically evolved mechanisms
genome quadrant duplication; silencing

Interclonal:
Promiscuous recombination — not all mechanisms are known.
Conjugation — dozens of species
Viral transduction and lysogenic integration: universal
Classical: phage borne toxins in *C. diphtheriae*
Plasmid interchange (by any of above) and integration
Toxins of *B. anthracis*
Pasteur: heat attenuation: plasmid loss; chemically induced
RNA viral reassortment; ?? and recombination?
Transgressive — across all boundaries: a World-Wide-Web
Artificial gene splicing
Bacteria and viruses have picked up host genes
(antigenic masking?)
Interkingdom: *P. tumefaciens* and plants
E. coli and yeast
Tobacco and immunocytes
Vegetable and mineral! oligonucleotides and yeast.

Host-parasite co-evolution:
Co-adaptation to mutualism or accentuation of virulence?
Probably divergent phenomena, with short term flareups and
Pyrrhic victories, atop long term trend to co-adaptation

Therefore, our evolutionary capability may be dismissed as almost totally inconsequential. In the race against microbial genes, our best weapon is our wits, not natural selection on our genes.

New mechanisms of genetic plasticity of one microbe species or another are uncovered almost daily (table 3). Spontaneous mutation is just the beginning. We are also dealing with very large populations, living in a sea of mutagenic influences (e.g., sunlight). Haploid microbes can immediately express their genetic variations. They have a wide range of repair mechanisms, themselves subject to genetic control. Some strains are highly mutable by not repairing their DNA; others are relatively more stable. They are extraordinarily flexible in responding to environmental stresses (e.g.,pathogens' re-

sponses to antibodies, saprophytes' responses to new environments). Mechanisms proliferate whereby bacteria and viruses exchange genetic material quite promiscuously. Plasmids now spread throughout the microbial world.[3] They can cross the boundaries of yeast and bacteria. Lateral transfer is very important in the evolution of microorganisms. Their pathogenicity, their toxicity, their antibiotic resistance do not rely exclusively on evolution within a single clonal proliferation.

We have a very powerful theoretical basis whereby the application of selective pressure (e.g., antibiotics in food animals) will result in drug resistance carried by plasmids or pathogens attacking humans. It is not easy to get direct and immediate epidemiologic evidence, but the foundations for these phenomena exist and must be taken into account in the development of policies. We have barely begun to study the responses of microorganisms under stress, although we have examples where root mechanisms of adaptive mutability are themselves responses to stress. In recent experiments, bacterial restriction systems are more permissive of the introduction of foreign DNA, possibly letting down their guard in response to "mutate or die" circumstances. This does not reflect bacterial intelligence that they know exactly what mutations they should undergo in response to environmental situations. Their intrinsic mutability and capacity to exchange genetic information without knowing what it is going to be is not a constant; it is certainly under genetic control and in some circumstances varies with the stress under which the microbes are placed.

Evolution is more or less proportionate to the degree of genetic divergence among the different branches of the 3-tiered tree of life, with the archaeal branch, the eubacterial branch, and the eukaryotes (figure 2). The tree illustrates the small territory occupied by humans in the overall world of biodiversity. It shows mitochondria right next to *Escherichia coli*. Bacterial invasion of a primitive eukaryote 2-1/2 to 3 billion years ago, synchronised with the development of primitive green oxygen-generating plants, conferred a selective advantage to complexes that could use oxygen in respiration. Our ancestors were once invaded by an oxidative-capable bacterium that we now call a mitochondrium and that is present in every cell of every body and almost every species of eukaryote. We did not evolve in a monotonous treelike development; we are also the resynthesis of components of genetic development that diverged as far as the bacteria and were reincorporated into the mitochondrial part of our overall genome. Another example of lateral transfer is the symbiosis that resulted from chloroplast invasion of green plants.

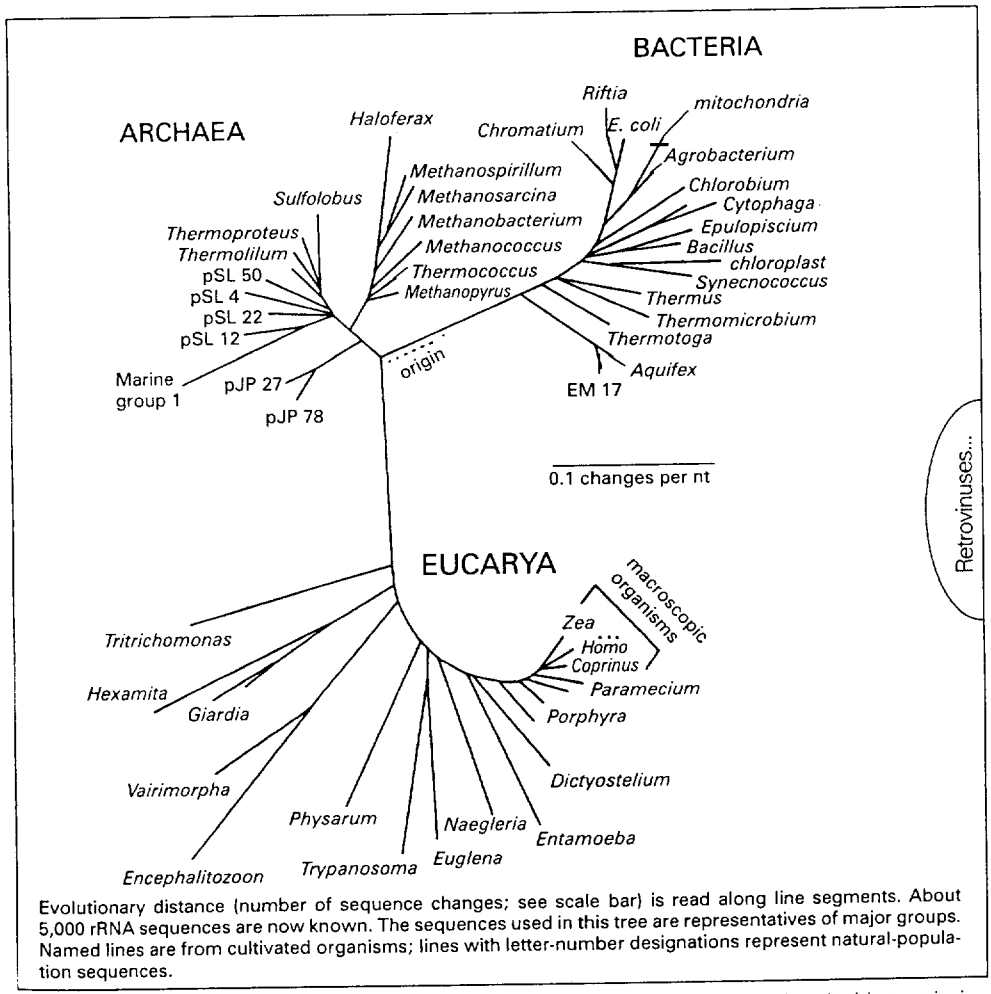
The outcome of encounters between mutually antagonistic organisms is intrinsically unpredictable. The 1918 influenza outbreak killed half percent of the human population; but because the consequences were to either kill the host or leave the host immune, the virus died out totally, leaving no trace in our genomes, as far as we know. Historic serology on survivors has found memory cells and antibodies against H1N1, the serotype of the resurrected 1918 virus. Unlike the influenza virus, which left no known genetic imprint, 400 to 500 retroviruses are integrated into our human genome. The full phylogeny of these encounters is unknown, but many of these viruses may precede the separation of homo sapiens from the rest of the hominid line.

Infectious agent outcomes range from mutual annihilation to mutual integration and resynthesis of a new species. Much has been made of the fact that zoonoses are often more lethal to humans than to their original host, but this phenomenon cannot necessarily be generalised. Most zoonoses do not affect humans adversely. Some are equally capable in a new host. We tend to pay most attention, however, to those, such as yellow fever, for which we have not genetically or serologically adapted and which cause severe disease.

Canine distemper provides an example of a quasihereditary adaptation. In the

Serengeti, the disease migrated from village dogs to jackals, which shared prey and had contact with lions. About one-fourth of the preserve's 4,000 lions died of canine distemper[4] but the survivors are immune and will pass immunoglobulin to their offspring. The cubs' maternal immunity will likely mitigate infection and permit a new equilibrium, not because of genetic adaptation but because of the preimmunised host. This is also the most plausible explanation for how savage the polio virus has been as a paralytic infection of young people. It may also apply to hepatitis, where cleaner is not always better if it means we do not have the "street smarts" to respond to new infectious challenges. These nongenetic adaptations between parasite and host complicate our outcome expectations.

Short-term shifts in equilibrium can give ferocious but temporary advantages to a virus. Long-term outcomes are most stable when they involve some degree of mutual accommodation, with both surviving longer. New short-term deviants, however, can disrupt this equilibrium. The final outcome of the HIV pandemic cannot be predicted. More



Evolutionary distance (number of sequence changes; see scale bar) is read along line segments. About 5,000 rRNA sequences are now known. The sequences used in this tree are representatives of major groups. Named lines are from cultivated organisms; lines with letter-number designations represent natural-population sequences.

Figure 2. The 3-domain tree of life based on small-subnit rRNA sequences. Reprinted with permission of Norman R. Pace and ASM News. ASM News 1996;62(9):464.

Table 4. The origin of viruses

Viruses are genomic fragments that can replicate only in the context of an intact living cell. They cannot therefore be primitive antecedents of cells. Within a given species, viruses may have emerged as genetic fragments or reduced versions from chromosomes, plasmids, or RNA of 1) the host or related species 2) distant species 3) larger parasites of the same or different hosts 4) further evolution and genetic interchange among existing viruses Once established, they may then cycle back into the genome of the host as an integrated episome; there they may have genetic functions or in principle might re-emerge as new viruses. These cycles have some substantiation in the world of bacterial viruses; but we have no clear data on the provenience of plant or animal viruses.

strains with longer latency may be taking over, mitigating the disease. However, deviant strains could counteract this effect by overcoming immunity and rapidly proliferating, with earlier and more lethal consequences.

We should also consider somatic evolution, a Darwinian process that occurs with every infection. In the clonal selection model of immunogenesis,[5] an apparently random production of immunoglobulin variants, both by reassortment of parts and by localised mutagenesis, gives rise to candidate antibodies, which then proliferate in response to matching epitopes. We do not understand the details of how a given epitope enhances stepwise improvements in affinity and productivity of antibodies at various stages. The process may be more complicated than we realise; so may Darwinian evolution.

Despite the prior arguments against relying on host or genotype evolution as a response to infection, historically we have done so and now have "scars of experience." A notable example is malaria, wherein the Duffy mutation against *Plasmodium vivax* is the only host defence with no deleterious consequences. The thalassaemias, G6PD deficiency, and haemoglobin S are all haemopoietic modifications that thwart the plasmodia; but in homozygotes, they themselves cause disease. In the evolution of our species, for every child spared an early death because a haemoglobin S mutation impeded Plasmodium development, another will succumb to sickle cell disease unless we can intervene. Specific remedies do not exist. Although somatic gene therapy is an interesting possibility, one that will probably progress in the next 20 years, it is paradoxical that we know more about haemoglobin S than any other molecular disease. The entire concept of genetic determination of protein structure has been based on these early observations, yet we are still searching with limited success for ways to put it to therapeutic use.

Biotechnology may enable other forms of genetic intervention through which homo sapiens could conceivably bypass natural selection and random variation. In the absence of alternatives, we might speculate about these kinds of "aversive therapies" as a last resort to save our species.

The ultimate origin of life is still the subject of many theories, as is the origin of viruses (table 4). Each virus is different. We know nothing of virus phylogenies and cannot even substantiate the distinctions of the several hundred categories. We do not know their origin, only that they interact with host genomes in many ways. Particles could come out of any genome, become free-living (i.e., independent, autonomously replicating units in host cells), re-enter a host genome as retroviruses and possibly oth-

Table 5. TECHNOLOGY The good news!

Antibacterial chemotherapy
Dilemmas of regulation of (ab) use.
Antiviral chemotherapy
Much more difficult problem, inherently.
Vaccines
Vaccination as service to the herd!
NEW approaches: hot biotechnology is coming along
Immunoglobulins and their progeny
Phage display and diversification: biosynthetic antibody
Passive immunisation for therapy
Biological response modifiers
New world of interleukins, cell growth factors
Technologies for diagnosis and monitoring
Homely technologies needed:
simple, effective face-masks
palatable water-disinfectants
home-use diagnostics of contamination

ers do, and repeat the cycle dozens of times. But no one can give a single example or claim to have significant knowledge of how any particular virus evolved, thus presenting a scientific challenge for the next 20 or 30 years.

We are dealing with more than just predation and competition. We are dealing with a very complicated coevolutionary process, involving merger, union, bifurcation, and reemergence of new species. Divergent phenomena can occur in any binary association, with unpredictable outcomes. We have hundreds of retroviruses in our genome and no knowledge of how they got there. As to HIV, we have no evidence as yet that it has ever entered anyone's germ line genome: we really do not know whether it ever enters germ cells. The outcomes of even that interaction could be much more complicated than the purely parasite/host relationships we are accustomed to.

Innovative technologies for dealing with microbial threats have the potential for fascinating therapeutic opportunities (table 5). Some, like bacteriophage, have been set aside as laboratory curiosities. Nothing is more exciting than unraveling the details of pathogenesis. Having the full genomes of half a dozen parasitic organisms opens up new opportunities for therapeutic invention in ways that we could not have dreamed of even 5 years ago, which will lead to many more technologies. In food microbiology, we should keep in mind the probiotic as well as the adversarial and pathogenetic opportunities in our alimentary tracts.

The Committee on International Science Engineering and Technology report[6] provides some recommendations (table 6). We need a global perspective. We need to invest in public health, not just medical care, in dealing with disease.

Today we emphasise individual rights over community needs more than we did 50 to 75 years ago. Restraining the rights and freedoms of individuals is a far greater sin than allowing the infection of others. The restraints placed on Typhoid Mary might not be acceptable today, when some would prefer to give her unlimited rein to infect others,

Table 6. What's to be done?

1. Concerted global surveillance and diagnosis of disease outbreaks and endemic occurrence.
2. Vector management and provision for safe water and food supplies; and assurance of adequate nutrition.
3. Public and professional education.
4. Scientific research on causes of disease, pathogenic mechanisms, bodily defences, vaccines and antibiotics.
5. Sharing and provision of the technical fruits of such research.

with litigation their only recourse. In the triumph of individual rights, the public health perspective has had an uphill struggle in recent pandemics.

Education, however, is a universally accepted counter measure, especially important in foodborne diseases. Food safety programmes should more specifically target food handlers, examining their hands to determine if they are carriers, to ensure they are complying with basic sanitation.

We typically do this only after an outbreak. Perhaps we should have further debate on the social context for constraints and persuasion to contain the spread of infectious agents.

References

1. *Webster RG, Bean WJ, Gorman OT, Chambers TM, and Kawaoka Y. Evolution and ecology of influenza A viruses.* Microbiological Reviews *1996;**56**:152-179.*
2. *Taubenberger JK, Reid AH, Frafft AE, Bijwaard KE, Fanning TG. Initial genetic characterisation of the 1918 "Spanish" influenza virus.* Science *1997;**275**:1793-1796.*
3. *Lederberg J. Plasmid (1952-1997).* Plasmid *1998;**9**:1-9.*
4. *Roelke-Parker ME, Munson L, Packer C, Kock R, Cleaveland S, Carpenter M, et al. A canine distemper virus epidemic in Serengeti lions (Panthera leo).* Nature *1996;**379**:441-445.*
5. *Lederberg J. The ontogeny of the clonal selection theory of antibody formation: reflections on Darwin and Ehrlich.* Ann N Y Acad Sci *1988;**546**:175-187.*
6. *NSTC-CISET Working Group on Emerging and Reemerging Infectious Diseases. Infectious disease:a global health threat. Washington (DC): The Group;1996.*