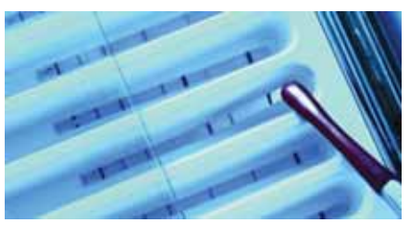
Fifty years of microbiology, antibiotics and vaccines

Microbiology has advanced enormously over the past 50 years. First impressions of a routine microbiology laboratory suggest that the microscope, Bunsen burner and incubator continue to have a major part to play; however, on closer inspection, great changes can be identified, as Trevor Winstanley explains.



Reverse hybridisation using DNA strip technology

Traditionally, clinical microbiology has been labour-intensive, slow and, to a great extent, confirmatory. Originally, most laboratories prepared their own media and developed their own methods to isolate bacteria, which, in consequence, sometimes generated variable results. Over the years, culture media have improved, becoming more defined and standardised. However, even though chromogenic substrates have been introduced, results are still not available in the same timeframe as those produced by other disciplines.

Traditional microbiological methods rely on the culture of potential pathogens from clinical samples, which usually takes 18–48 hours, and identification using colonial morphology and biochemical end-products of metabolism, and then determination of their susceptibility or resistance to a range of antibiotics.

The diagnostic capabilities of modern laboratories have improved in response to the technological revolution in molecular biology and complementary automation, allowing the detection of microbial DNA and RNA without the need to culture the organisms. Laboratories have also changed their focus (eg blood science laboratories) with staffing patterns, workflow and turnaround times all being modified. Technological advances have even permitted some aspects of microbiology to be undertaken in real time.

Advances in antimicrobial therapy, vaccine development and the enforcement of antiseptic and hygienic principles, as well as the prevention of crop disease and study of heredity and molecular biology, have been introduced to positive effect. Life expectancy has doubled and morbidity caused by infectious diseases has decreased dramatically.

The detection of *Mycobacterium tuberculosis* illustrates these points well. At the start of the author's 30-year career in microbiology, TB media were so poor that guinea-pig inoculation was the most sensitive option to detect infection. Over the years, media have improved (eg Kirchner and Middlebrook 7H10) and isolation times have been reduced from 6–12 weeks to 10 days, as a result of the use of rapid liquid culture systems on automated machines.

Now, positive cultures can be examined by reverse hybridisation of polymerase chain reaction (PCR) products to complementary probes immobilised on membrane strips (eg HAIN technology). Identification and

TIMELINE OF PATHOGEN DISCOVERY 1957–2007

1957

Pandemic of Asian flu started in SW China and spreads through the Pacific

Spongiform encephalopathy (kuru) identified in cannibal Fore tribe of Papua New Guinea

1958

Argentine haemorrhagic fever virus isolated

1959

Lassa fever described in Nigeria

1960

Coxiella burnetti (Q-fever) isolated

Staphylococcus aureus as a major cause of hospital infection

CMV isolated

1964

Australia antigen detected

Epstein-Barr virus discovered – first to be associated with cancer

1967

Marburg disease reported in Angola

1968

Pandemic of 'Hong Kong flu'

Mice infected by airborne transmission of influenza virus

1969

The Lassa arenavirus is isolated

1970

Reverse transcriptase is discovered

1973

Rotavirus discovered

1974

Transmission of Creutzfeldt-Jakob disease to humans

1975

Seventh pandemic of cholera in Indonesia

Lyme disease characterised

Parvovirus B19 discovered

1976

Outbreak of Legionnaire's disease in Philadelphia, USA

Cryptosporidium parvum identified

Swine flu outbreak starts in New Jersey, USA

Ebola virus emerges in Sudan and Zaire (290 deaths from 318 cases)

LANDMARKS IN MOLECULAR BIOLOGY.

1938	Term 'molecular biology' first used
1941	Term 'genetic engineering' first used
1944	Avery shows that DNA carries genetic information
1946	First genetic recombination experiments
1947	McClintock discovers transposable elements
1953	Classic *Nature* double-helix publication by Watson and Crick
1956	Kornberg discovers DNA polymerase
1958	DNA made in a test tube
1960	Hybrid DNA-RNA molecules created
	Messenger RNA discovered
1966	Genetic code is cracked
1970	Restriction enzymes discovered
1971	First gene completely synthesised
1973	Recombinant DNA technology using restriction enzymes and ligases
1975	Monoclonal antibodies produced
	Southern blotting developed
1977	Methods to determine DNA sequences
1980	Human interferon produced in genetically engineered bacteria
1986	Polymerase chain reaction (Kary Mullis)
	Reverse transcriptase PCR (RT-PCR) for amplification of RNA
1993	Taqman
1995	*Haemophilus influenzae* genome is sequenced
1996	Molecular beacons
1998	LightCycler
2004	Human genome sequenced

simultaneous detection of resistance genes also can be performed for *M. tuberculosis* complex, with direct detection of the microorganism from the sample now achievable using this methodology.

The 100-year-old tuberculin skin test for TB infection, which took between three and seven days to interpret, is also being superseded by more rapid manual and automated blood tests based on detection of T cells reactive to *M. tuberculosis* proteins. The increased specificity of these tests now also permits detection of latent infections in the asymptomatic population.

Screening for methicillin-resistant *Staphylococcus aureus* (MRSA), an increasing requirement in most hospitals, was traditionally culture-based, took 18–24 hours and often did not impact on patient management. Now, a variety of technologies including latex agglutination, bioluminescence, PCR and other probe systems are being used to detect the *mec*A gene or the protein it encodes. To increase specificity, organism-specific *S. aureus* sequences are also sought (eg *fem*A, *nuc*, toxin or coagulase genes). The most specific PCR methods use Molecular Beacons to detect staphylococcal *SCCmec* sequences and *orfX* genes, and the latest systems are capable of detecting MRSA in mixed culture in two to three hours.

Automation was slow to arrive in microbiology; however, blood cultures are now virtually always read by automated instruments. Additionally, disc-diffusion or agar-incorporation breakpoint antibiotic susceptibility plates are conveniently read by video camera (eg mastascanelite). Many laboratories have introduced automated antibiotic susceptibility and identification machines using microbroth methods, which are capable of generating results in four to six hours.

Drug assays on body fluids are now performed by techniques such as fluorescence polarisation immunoassay, while even traditional microscopy is being replaced by fluorescence flow cytometry and cluster analysis. Other traditional methods may also become mechanised as commercial companies provide automated plate ordering systems.

Despite these developments, the simple 50-year-old antibiotic disc remains the most commonly used product for susceptibility testing worldwide, and continues to be useful in laboratories which use automated systems that cannot process certain fastidious organisms.

Microbiology laboratories are now familiar with immunochromatographic assay technology and use it to detect rotavirus, respiratory syncitial virus (RSV), *Clostridium difficile* toxins, malaria, *Legionella* antigens and more. Microarrays have the potential to revolutionise pathogen detection further, allowing multiple simultaneous organism detection. Real-time PCR technology is now capable of detecting and identifying within just a few hours the 25 most important bacterial and fungal species that cause bloodstream infections. Matrix-assisted laser desorption/ionisation–time of flight (MALDI-TOF) mass spectrometry and pyrosequencing will soon become tools of the diagnostic laboratory.

MISPLACED OPTIMISM

In the late 1960s, the introduction of effective vaccines and antibiotics led us to believe that infectious diseases had been conquered.

TIMELINE OF PATHOGEN DISCOVERY 1957–2007

1977

Legionella pneumophila identified

1978

Hantavirus (Sin Nombre virus) discovered

Clostridium difficile first identified as cause of pseudomembranous colitis

1979

Campylobacter jejuni identified as enteric pathogen

1980

S. aureus causes toxic shock syndrome

Human T-lymphotropic virus 1 (HTLV-1), cause of T-cell lymphoma/leukaemia, discovered

1981

Acquired immune deficiency syndrome (AIDS) recognised by CDC

First human case of Puumala virus (haemorrhagic fever)

1982

Borrelia burgdorferi isolated from *Ixodes* tick

E.coli 0157:H7, haemorrhagic colitis and haemolytic uraemic syndrome.

500 cases, 20 deaths in Wishaw, Scotland

Human T-lymphotropic virus II (HTLV-II), cause of hairy-cell leukaemia, is discovered

Prions described

First human case of Seoul virus (haemorrhagic fever)

1983

Helicobacter pylori shown to be the cause of peptic ulcers

Discovery of the human immunodeficiency virus (HIV)

First human case of rotavirus B

First human case of HIV1

1984

First human case of *Scedosporium prolificans*

In 1967, William Stewart, the US Surgeon General, said, perhaps unwisely, that is was "time to close the book on infectious diseases, declare the war against pestilence won and shift national resources to such chronic problems as cancer and heart disease." In 1978, the United Nations adopted a resolution to eradicate infectious diseases by the year 2000. Smallpox has already been eradicated from the world. In the last decade, total reported cases of many other infections showed evidence of decrease, including poliomyelitis, infections with meningococcus C and pneumococcus, human immunodeficiency virus (HIV) and hepatitis C virus (HCV) infections in developed countries, diphtheria and cholera.

Vaccination programmes should eradicate poliomyelitis from the world within the next few years. Conjugate pneumococcal vaccine has decreased dramatically the incidence of invasive disease and conjugate meningococcal C vaccine should ensure that bacterial meningitis is eliminated once vaccines against serotypes A, C, Y, W135 and B come online in the next decade. The sequencing of the HCV genome in 1987 has enabled bloodborne spread to be brought under control and highly active antiretroviral therapy (HAART) has transformed the nature of HIV disease.

However, three diseases (tuberculosis, HIV infection and malaria) account for 50% of worldwide mortality, and, despite the advances highlighted above, we have made little impact on them. There are eight million new cases of TB every year and, even in the developed world, cholera and diarrhoeal disease can still present a devastating problem.

A TIMELINE FOR THE DEVELOPMENT OF ANTIBIOTIC RESISTANCE.

1959	50% penicillin resistance in *S. aureus*
1961	MRSA first isolated in UK
1964	First transmissible β-lactamase (*E. coli* TEM1)
1967	Low-level penicillin-resistant pneumococci in Australia and Papua New Guinea
1970	TEM-2 β-lactamase
1972	Ampicillin-resistance due to β-lactamase (TEM) in *H. influenzae*
1976	Penicillinase-producing (TEM) *N. gonorrhoeae* isolated
1978	High-level penicillin-resistant pneumococci in South Africa
	First high-level aminoglycoside-resistant enterococcus
1983	First ESBL (SHV-derived, Germany)
1986	First case of vancomycin-resistant enterococci (France)
1987	First TEM-derived ESBL (TEM3)
1988	First plasmidic AmpC β-lactamase
	First *Plasmodium falciparum* resistant to chloroquine *in vivo*
1989	First CTX-M ESBL (*E. coli* isolate in Germany)
1990	Multidrug-resistant TB (MDRTB) in New York and Florida
1992	Isolation of *N. gonorrhoeae* with reduced susceptibility to fluoroquinolones
1996	Vancomycin-intermediate *S. aureus* (VISA) isolated in Japan
1997	hetero-VISA isolated in Japan
1998	Plasmid-mediated resistance to fluoroquinolones in *E. coli*
2001	Clonal outbreak of CTX-M ESBL in UK
2002	Vancomycin-resistant *S. aureus* (VRSA) isolated in USA
2004	Deaths due to CTX-M ESBL-producing *E. coli* in Telford, UK
	Emerging carbapenem resistance in β-lactam-, aminoglycoside- and fluoroquinolone-resistant *Acinetobacter* spp.

THE GENOMICS ERA

Undoubtedly, molecular biology has had a massive impact on clinical microbiology over the past 10–15 years, although much progress was achieved prior to this. The development of genetics, specifically molecular genetics, resulted in a revolution that has led to a greater understanding of the basic mechanisms of disease-causing pathogens, and our initial understanding of gene function. The past 50 years have seen massive leaps in technology, with molecular biology maturing into the separate fields of genomics and proteomics, revealing the secrets of DNA replication, RNA transcription and protein translation.

Little was known about the structure of DNA in the early 1950s; however, manipulation and amplification of specific strands of DNA was achievable by the late 1980s. Now, PCR has been developed further to provide real-time detection methods using Taqman, Molecular Beacon and LightCycler chemistries.

Academia was swift to embrace these technologies and, as a result, we now have a better understanding of many infectious diseases. New vaccines have been developed and recent genome sequencing projects have provided data on unknown genes and also information relating to the function of known genes. However, PCR is not available in every microbiology laboratory, although its uses and benefits are proven. Acceptance of molecular biology will follow when cost restraints are overcome, when there is adequate training and when methods are available in commercial kit form (eg DNA hybridisation strip tests).

Ten years ago, microbiologists cloned and sequenced one gene at a time. Now, technology and experience have intervened

TIMELINE OF PATHOGEN DISCOVERY 1957–2007

1985

Enterocytozoon bieneusi shown to cause persistent diarrhoea

1986

Cyclospora cayetanensis found to be a cause of persistent diarrhoea

First human case of rotavirus C

First human case of HIV2 (AIDS)

1987

First human case of Banna virus

1988

Human herpes virus 6 (HHV-6) identified as a cause of roseola subitum

Hepatitis E, a foodborne hepatitis, identified

First human case of picobirnavirus (gastroenteritis)

First human case of Barmah Forest virus

1989

Ehrlichia chafeensis identified

Hepatitis C causing liver infection is identified

First human case of *Corynebacterium amycolatum* (endocarditis)

1990

Bartonella henselae identified as cause of cat scratch disease (bacillary angiomatosis)

1991

Encephalitozoon hellem shown to be associated with conjunctivitis and disseminated disease

Guanarito virus shown to be cause of Venezuelan haemorrhagic fever

New species of *Babesia* shown to be the cause of atypical babesiosis

1992

New strain of epidemic cholera, *Vibrio cholerae* O139, identified

1993

Tropheryma whipplei discovered and named as the cause of Whipple's disease

Encephalitozoon cuniculi shown to be a cause of disseminated disease

Encephalitozoon intestinalis shown to be a cause of microsporidiosis

First human case of Sin Nombre virus (Hantavirus pulmonary syndrome)

First human case of *Gymnophalloides seoi* (gastrointestinal disease)

First human case of *Rochalimaea elizabethae* (endocarditis, bacteraemia)

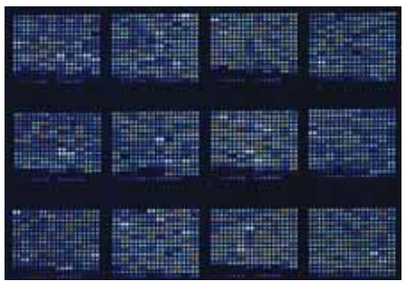
to allow much more rapid progress. Craig Venter, David Hamilton-Smith and Claire Fraser sequenced the complete genome of *Haemophilus influenzae* in 1995, while the genome of the SARS virus was sequenced within a month of its emergence and identification. We now have access to over 200 bacterial genomes, 1600 viral genomes, the malarial parasite genome and even the human genome. Soon, genome sequencing will provide whole-genome answers.

DISCOVERY OF NEW PATHOGENS

New organisms continue to be discovered as a result of improvements in technology and the application of genomics. In the latter half of the 20th century, multiple approaches to microbial identification were used and we became optimistic about the possibility of detecting all known pathogens. Examples include the following:

- HIV was discovered in 1983 by classical isolation and corroboration by detection of reverse transcriptase
- *Helicobacter pylori* was identified by culture, microscopy and self-inoculation
- human papillomavirus (HPV) types 16 and 18 in cervical carcinoma were identified by DNA hybridisation
- using PCR, the organism causing bacillary angiomatosis, *Bartonella henselae*, was identified in 1990, and the organism responsible for Whipple's disease, *Tropheryma whipplei,* was identified in 1993
- consensus PCR was used to identify Sin Nombre virus in 1993
- representation difference analysis was used to identify the causative agent of Kaposi's sarcoma, HHV8, in 1995
- molecular technology was vital in identifying the important metapneumovirus and, in 2007, *Bartonella rochalimae*, a previously unknown pathogen from Peru.

In total, 1407 human pathogen species are currently recognised. Of these, 177 (13%) are regarded as emerging or re-emerging and, of this subset, 38 (21%) were first recognised during the past 25 years, corresponding to a rate of three new species recognised every two years. These new species could, under the right conditions, recombine, jump species and generate new human pathogens.



Multiple analyses of nucleic acids by microarray technology.

Emerging or re-emerging pathogen species disproportionately are viruses (disproportionately RNA viruses) with newly recognised species. Some degree of association has been identified with particular transmission routes, most notably (but not exclusively) foodborne and faecal–oral transmission.

Most emerging infectious diseases are zoonoses, some examples of which are listed below:

- HIV
- variant Creutzfeldt-Jakob disease (vCJD)
- Hantavirus
- *Cryptosporidium parvum*
- Nipah virus of pigs
- Hendra respiratory virus of racehorses
- the threat from xenotransplantation (porcine endogenous retroviruses)
- Sin Nombre virus (Hantavirus) in south-west USA spread by rodent excrement and bites

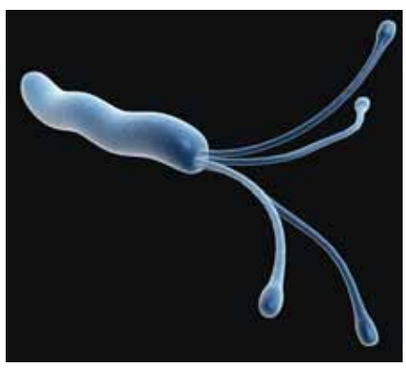
Avian influenza, the causative agent for recent outbreaks in Hong Kong, Thailand and Vietnam, has been controlled by culling fowl, but the number of outbreaks, and the fact that the genome readily changes (H5N1 → H9N2), suggests that a human pandemic could occur.

Severe acute respiratory syndrome (SARS) appeared in 2003 when the coronavirus spread from wild food animals in Asian markets to humans. Other human infections have resulted from contact with animals. For example: Ebola originated in primates, vCJD spread following consumption of the bovine spongiform encephalitis (BSE) prion in beef, and West Nile flavivirus is transferred from birds to mammals by the urban mosquito, and is spread among human populations via intercontinental air flights.

The most important drivers of pathogen emergence and re-emergence tend to be changes in the non-human environment (ie land use and agriculture) and the human environment (ie society and demography).

Advances in medicine have resulted in extensive niches for infection. Today's surgery is much more adventurous, and more effective treatments for some cancers have resulted in improving and increasing survival rates. Advances in physiotherapy, antibiotic treatment and digestion ensure that cystic fibrosis patients survive well into adulthood. Heart-lung, stem cell and bone marrow transplantations are now commonplace, the consequence of which is an increasing number of immunosuppressed patients. Some patients are also compromised by co-infection (eg with HIV).

Globalisation is a phenomenon that has an impact on more than just the business



Helicobacter pylori, the cause of peptic ulceration.

TIMELINE OF PATHOGEN DISCOVERY 1957–2007

1994

Sabia virus shown to be cause of Brazilian haemorrhagic fever

First human case of Hendra virus (ARDS, encephalitis)

First human case of Bagazo virus (Spondweni fever)

Anaplasma phagocytophilum (human granulocytic anaplasmosis) discovered

1995

Childhood diabetes shown to be related to infection with coxsackie B virus

Human herpes virus 8 (HHV-8) shown to be associated with Kaposi's sarcoma in AIDS patients

Hepatitis G virus (hepatitis) discovered

1996

First human case of Australian bat lyssavirus (bat rabies)

First human case of trematode *Metorchis conjunctus*

First human case *Trachipleistophora hominis* (microsporidiosis)

First human case of variant Creutzfeldt-Jakob disease

First human case pf Andes virus (Hantavirus pulmonary syndrome)

Vibrio parahaemolyticus O3K6 (pandemic gastroenteritis)

1997

Resurgence of diphtheria in Russia

Avian influenza H5N1 transfers to Hong Kong citizens, a third of whom die

First human case of Laguna Negra virus (Hantavirus pulmonary syndrome)

First human case of TT virus (hepatitis, possible)

1998

First human case of Menangle virus

First human case of *Brachiola vesicularum* (microsporidiosis)

As a result of climate change, cases of mosquito-borne disease are increasing.

environment. The equilibrium between microbes and man is disturbed continually by the impact of human behaviour. Increased global populations, intensive animal farming and food production, sexual practices, poverty, global warming and the breakdown of public health measures all have an effect on the incidence of infectious disease. Historically, plague, measles and syphilis have been associated with travel; however, nowadays West Nile encephalitis, dengue and antibiotic-resistant organisms increasingly are transmitted in this manner. Vectors also are important, as the following two examples demonstrate.

- In 1985, tyres imported into Texas from Asia spread the Asian tiger mosquito and transmitted dengue fever.
- In 2000, New York was affected by bird- and mosquito-borne West Nile encephalitis.

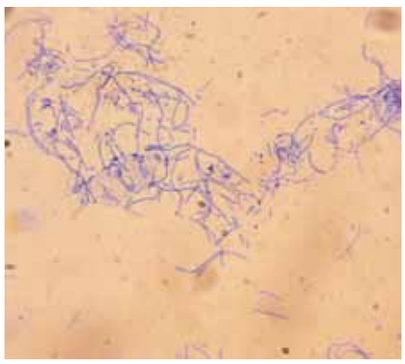
Global warming has altered the ecosystem, resulting in warmer summers, milder winters and higher sea levels. If the range of vector- and waterborne infections alter, new zoonoses are likely to emerge.

The use of bacterial agents to inflict harm has also entered the realms of modern warfare. The US Centers for Disease Control and Prevention (CDC) has identified 37 bacterial agents and toxins as viable terrorist threats with the capacity to be engineered for greater impact. Such biological agents are capable of being manipulated to increase their stability, allowing them to be produced in large quantities, and be capable of being sprayed. In particular, the agents of anthrax, plague, smallpox, *Clostridium botulinum, Coxiella burnetti, Francisella tularensis, Brucella suis* and haemorrhagic fever viruses represent serious threats to human health, as they are difficult to identify quickly, resistant to antibiotics, not protected by immunisation and result in a high mortality rate.

ANTIBIOTICS

Over the past 50 years, methodology for antibiotic discovery has evolved from screening for natural antibiotics, screening fermentation products and selective inhibition of biochemical targets to 'me too' improvements. Over the past 10 years, less than a dozen significant antibacterial agents have emerged. Large pharmaceutical companies are losing interest in antibiotics because they may become reserved/restricted, are likely to select resistance, will be used in short courses, and have small markets. In response, small biotechnology companies have emerged with an interest in antibacterial discovery, and large pharmaceutical companies may look to these as a source of antibiotic development.

Over the period from 1998 to 2003 only nine new antibiotics (rifapentine, cefditoren, linezolid, quinupristin/dalfopristin, moxifloxacin, gatifloxacin, ertapenem, gemifloxacin and daptomycin) were approved, of which only linezolid and daptomycin have a novel mechanism. Of 315 novel medical entities (NMEs) in disclosed pipelines of the 15 largest pharmaceutical companies, only five (1.6%) were antibacterial agents. This reflects stricter standards for equivalence, the risk of rapidly emerging resistance, a clinical preference for narrow-spectrum agents, a varying regulatory climate, a relative high purchase price, and increased post-marketing surveillance.



Bacillus anthracis, the causative agent of anthrax.

The availability of new technologies such as genomics has opened up new approaches for antibacterial research. A genomic approach had been proposed to enable improvements to be made to existing compounds in terms of their efficacy or safety, to understand synergy and antagonism, to improve pharmacokinetics and to avoid resistance. In general, however, the genomic approach to antibiotic discovery has been unsuccessful, due to problems with synthesis, pharmacokinetics or impermeability and efflux.

ANTIBIOTIC RESISTANCE

Bacterial resistance to antibiotics is now a major issue for clinical microbiology. The emergence of drug-resistant pathogens could be a disaster in the making if antibiotic discovery does not progress. We are moving steadily towards the post-antibiotic era, with resistance in a variety of bacterial species, as the following list demonstrates:

- methicillin resistance in *Staphylococcus aureus* (predominantly EMRSA-15 and -16)
- hetero- and frank glycopeptide resistance in *S. aureus* (GISA and GRSA)
- low- and high-level penicillin resistance in pneumococci (PRP)
- vancomycin resistance in enterococci (VRE)
- β-lactamase production in Enterobacteriaceae (chromosomal and now plasmidic AmpC, TEM- and SHV-derived ESBLs, CTX-M ESBLs acquired from *Kluyvera* spp.), haemophili and gonococci
- carbapenem resistance in *Klebsiella* with ESBLs and impermeability

TIMELINE OF PATHOGEN DISCOVERY 1957–2007

1999

Chlamydia shown to trigger an autoimmune disorder that can cause heart disease

First human isolate *Ehrlichia ewingii* (Ehrlichiosis)

First human case Nipah virus encephalitis

2000

Nipah virus kills 100 people in Malaysia, one million livestock killed

New York affected by bird- and mosquito-borne West Nile encephalitis

2001

CDC learns of first anthrax case – bioterrorism through the mail

First human case metapneumovirus

2002

First human case of *Cryptosporidium hominis*

Hyper-virulent *C. difficile* PCR ribotype O27 causes outbreaks

2003

WHO alerted to the threat of severe acute respiratory syndrome (SARS coronavirus) – the most effective response to an epidemic in history

Human monkeypox in the Western hemisphere

2004

Avian influenza H5N1 (bird flu) in Asia

C. difficile PCR ribotype O27 outbreak in Stoke Mandeville, UK

2006

Nosocomial transmission of Panton-Valentine leukocidin-positive community-acquired MRSA in UK (SCCmecIV)

2007

First human case of *Bartonella rochalimae*

- *Pseudomonas aeruginosa* (carbapenemases, efflux pumps, impermeability)
- pan-resistance through multiple mechanisms (β-lactamases including carbapenemases, aminoglycoside-modifying enzymes, gyrase mutations, changes in PBPs and OMPs (eg *Acinetobacter* spp.)
- multidrug resistance in *Mycobacterium tuberculosis* (MDRTB)
- acquired carbapenemase (class B: IMP, VIM and SPM metallo-β-lactamases; class D: OXA-23, -40 and -58 related; class A: KPC, SME and NMC/IMI) are rare but increasing, especially in non-fermenters.

ANTIVIRAL AGENTS

Mass screening of natural compounds has produced a number of antibacterial agents, but was not successful for antivirals. After the introduction of 5-iodo-2'-deoxyuridine in 1959, only four other antivirals were licensed over the years to 1990, and these were amantadine (influenza), ribavirin (RSV), acyclovir *et al.* (herpes) and AZT (HIV).

Subsequently, 35 new antivirals had been licensed by 2004, as a result of the use of genomics to identify and target enzymes such as retroviral reverse transcriptase, DNA- and RNA-dependent polymerases, helicases, proteases and neuraminidase. The use of genomics also enabled inhibitors to be engineered.

Mepron (atovaquone) for acquired immune deficiency syndrome (AIDS)-related pneumonia was introduced in the USA by Wellcome in 1992, and, in 1995, Valtrex (valaciclovir) was launched by Glaxo Wellcome as an anti-herpes successor to Zovirax (acyclovir). Zanamivir, approved in 1999, acts by blocking neuroaminidase, which prevents influenza virus from escaping the cell.

Fusion of the virus membrane with that of the host cell may also be inhibited, and small interfering RNAs (siRNAs) can trigger degradation of viral RNA products. High-affinity human monoclonal antibodies have the potential to fill the void left by the withdrawal of passive immunisation with sera.

VACCINES

Classical vaccine technologies have generated killed, live attenuated and subunit vaccines, all of which are incapable of addressing non-growing organisms (eg HCV, HPV types 16 and 18, *Mycobacterium leprae*) or hyper-variable organisms (eg meningococcus B, gonococcus, malaria and HIV). So-called reverse vaccinology is the genomic solution to these problems. Antigens likely to induce immunity are selected and those likely to induce autoimmunity are ignored. Genomics has revealed 29 such candidate antigens for meningococcus B.

T cells play a vital role in the control of HIV, malaria and some chronic diseases. Genetic manipulation has enabled the engineering of non-replicating viral vectors, replication-incompetent viruses and DNA vaccines to stimulate the production of such T cells.

A TIMELINE OF VACCINE DEVELOPMENT AND INTRODUCTION.

1955	Salk's killed virus polio vaccine
1957	Sabin's weakened live virus polio vaccine (OPV)
1958	World Health Organization (WHO) starts eradication of smallpox
1962	Oral polio vaccine (OPV) introduced
	Measles vaccine developed
1967	United Nations begins smallpox eradication programme
	Mumps vaccine developed
1970	First vaccination for rubella
1971	MMR licensed
1972	Routine smallpox vaccination in USA ends
1974	Smallpox eliminated from India
	First vaccine for chickenpox
	WHO targets tuberculosis, diphtheria, neonatal tetanus, whooping cough, poliomyelitis and measles. Later it adds yellow fever, hepatitis B and MMR vaccines
1977	Last 'wild' case of smallpox reported from Somalia
	14-valent pneumococcal vaccine developed
1978	Two laboratory-acquired cases of smallpox
1980	World Health Organization formally declares world free of smallpox
	First vaccine for hepatitis B
1981	SmithKline Beecham launches Energix-B hepatitis B vaccine, a genetically engineered recombinant vaccine
1991	Live oral attenuated *S. typhi* vaccine trialled
	H. influenzae b vaccine licensed
1992	SmithKline Beecham launches Havrix hepatitis A vaccine
1994	Americas declared polio-free
1995	Hepatitis B vaccine used in 28 countries
	Varicella and hepatitis A vaccines licensed
	First trials of DNA vaccines for HIV
1996	Acellular pertussis vaccine licensed for young infants
1999	Malaria vaccine developed by Altaf Lal of the CDC
	First HIV vaccine trial in Africa – weakened canarypox (three HIV genes)
	Rotavirus vaccine, licensed in 1998, withdrawn
2000	Measles declared non-endemic in USA
2001	USA plan to re-introduce smallpox vaccine vs. bioterrorism
2002	Lyme vaccine, licensed in 1998, taken off the market
2004	Inactivated flu vaccine recommended for infants 6–23 months old
2005	Rubella declared non-endemic in the USA
2006	First vaccine for human papillomavirus introduced

Genomic applications have revealed that the innate immune response recognises a signature of bacterial infection, and certain DNA, dsRNA or lipopolysaccharide sections stimulate Toll-like receptors and Nod proteins, acting as adjuvants to vaccines. Genomic vaccines are much safer than their early counterparts.

PANDORA'S BOX

The past 50 years have seen tremendous changes in microbiology. They have also witnessed improvements in antibacterial and antiviral therapy, as well as in vaccine development and infection control practices, all of which have contributed to significant improvements in human health and well-being.

Molecular biology has given us the key to microbiology's equivalent of Pandora's box; however, our worst enemy could be complacency. In bacteria, viruses and prions, we are faced with rapidly evolving enemies that continue to re-invent themselves and stay relentlessly one step ahead of our best endeavours. The next 50 years should be an even more interesting time. ■

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