Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

8

INFECTIOUS DISEASES

DIAGNOSTICS 193
Overview 193
Infectious Disease DNA Laboratory 196
Human Immunodeficiency Virus (HIV) 197
Transfusion-related Infections 199
PATHOGENESIS 201
Host Resistance 201
Drug Resistance 203
Virulence Factors 206
Cancer 206
EPIDEMIOLOGY 208
Antigenic Variation 208
Taxonomy 210
Nosocomial Infections 212
EMERGING INFECTIONS 213
Zoonoses 213
SARS 215
Bioterror 216
FUTURE 218
FURTHER READING 219

DIAGNOSTICS

OVERVIEW

Phenotypic-based Tests

The traditional or **phenotypic** methods for the detection of pathogens are well tried, relatively cheap and available in most hospital and private pathology laboratories (Table 8.1). However, their utility needs to be considered in the context that (1) Direct visualisation or culture is not always feasible. (2) These techniques can be time consuming and technically difficult. (3) Phenotypic variation can occur during a pathogen's life cycle; e.g., eggs, larvae and adult forms of a species may alter depending on the stage of development, the associated host or vector and whether the organism is free-living. Thus, antibodies or isoenzyme techniques for detection may become limiting and dependent on the stages in the life cycle. (4) Host immune responses can be delayed, or conversely, they remain persistent even after resolution of a previous infection. (5) Cross-reacting antibodies acquired from natural infections or vaccination can produce false positive results.

Phenotypic methods can be used to discriminate between isolates, genera and species. These approaches are less effective when it comes to distinguishing differences within species. More recently, the emergence of new pathogens, or the concern about bioterrorism, has brought an added urgency to the development of more efficient and rapid methods to detect pathogens and predict their potential virulence.

Genotypic-based Tests

Many of the traditional diagnostic tests are now being complemented or replaced by **genotypic** analysis, i.e., detection of DNA or RNA fragment sizes or specific sequences through nucleic acid hybridisation techniques, or increasingly today DNA amplification by the polymerase chain reaction (PCR). An expression frequently found in microbiologic DNA testing is **NAT** (nucleic acid amplification technique). NAT refers to a number of techniques apart from PCR that can be used to amplify DNA, e.g., ligase chain reaction. For convenience, the description "PCR" will be used interchangeably with "NAT" to indicate broadly based amplification of DNA. To synthesise or design a DNA probe or primer, it is necessary to sequence an organism's DNA to identify species-specific regions (see Table A.2, which provides more information about probes and primers). Today, whole genomes from many pathogens have been sequenced, and this information is available on databases. From this sequence (1) DNA primers can be

Table 8.1 Traditional diagnostic approaches for detecting pathogens

Method	Comments
Staining and visual identification under the naked eye or microscope (light or electron)	Characteristics such as colony size, shape and colour give a clue to the underlying organism. Using light microscopy and staining (e.g., Gram stain) is the most rapid approach to identifying many bacteria. Viruses are visible with the electron microscope.
Culture and growth	Organisms display culture and growth characteristics; e.g., *M. tuberculosis* is a slow grower. Organisms can be further distinguished by their growth in particular media; e.g., chocolate agar is needed for fastidious growers such as *H. influenzae*.
Biochemical characteristics	The presence of catalase (degrades hydrogen peroxide) is used to differentiate many gram positive organisms.
Immunological, i.e., recognition of antigenic determinants related to an organism and the host's specific immune responses to it (production of antibodies)	An antiserum can be used to agglutinate *Salmonella* and *Shigella* species. Antibodies produced in the patient can be used to identify both active and past infections.

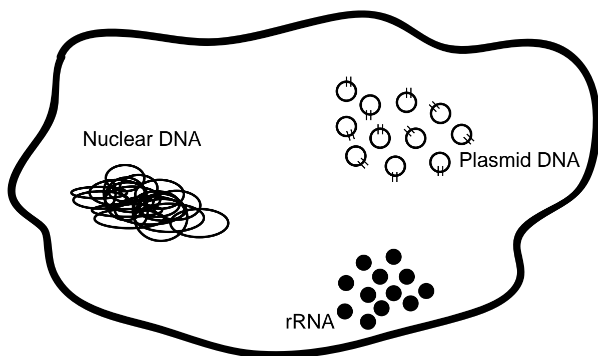

Fig. 8.1 Potential targets for nucleic acid (DNA or RNA) probes in pathogen detection.
Nuclear DNA or RNA and rRNA (ribosomal RNA) are ubiquitous and stable, providing useful targets for a wide range of hybridisation or PCR-based assays. Plasmid DNA can be present in multiple copies, although they can be lost or undergo rearrangements. Therefore, plasmid DNA is not a dependable target for testing but can provide information on infectivity or antibiotic resistance. Nuclear and rRNA probes provide wide-ranging genotypic assays extending across the entire taxonomic spectrum from Family to Genus to Species and Strain.

synthesised for PCR. (2) DNA probes can be isolated or synthesised (oligonucleotide probes) for nucleic acid hybridisation techniques.

Ribotyping: A useful target for a probe is repetitive DNA, an example of which is ribosomal RNA (rRNA). rRNA and the rRNA genes in chromosomal DNA provide naturally derived amplified products that enhance their hybridisation potential. In these circumstances, radio-labelled DNA probes can be avoided since the signal-to-noise ratio is increased because of the amplified target sequence (Figure 8.1). This method of differentiating between bacteria is called ribotyping. For ribotyping, DNA is broken into fragments using restriction endonucleases. DNA probes that are specific for rRNA genes, then allow various fragments to be identified and compared. This approach is very similar to DNA mapping or Southern blotting in eukaryotic DNA (see the Appendix), and the bacterial hybridisation patterns are comparable to eukaryote DNA polymorphisms.

Pulsed field gel electrophoresis (PFGE): This type of DNA electrophoresis is capable of separating very large DNA fragments. These fragments result from digesting the organism's genome with a restriction endonuclease enzyme such as *Not*I that cuts DNA very infrequently (see Table A.1). The pattern of the fragments generated in this way is sufficiently discriminatory to allow bacterial strains to be distinguished. Read more about PFGE under *Legionella*.

DNA hybridisation: 16S rRNA and 12S rRNA contain highly conserved regions common to all bacteria as well as highly variable regions unique to particular species. Therefore, multifunctional DNA or RNA probes can be prepared since they can be generic for all bacteria, or specific for different species or strains.

PCR

It is reasonable to say that whatever can be done by the various DNA probes or hybridisation techniques just described can also be accomplished, and probably better, with PCR. Thus, there are genus-specific and species-specific PCR-based assays and many other strategies appropriate for a range of activities involving microorganisms in the environment, food industry, research and clinical practice. Ultimately, the most information that can be obtained about a microorganism's DNA is through sequencing of its genome. This is now increasingly possible as a consequence of the Human Genome Project and the sequencing of many model

organisms (see Table 8.2). The spread of epidemics or hospital-acquired (nosocomial) infections will be followed and characterised with more accuracy by the identification of unique DNA fingerprints for individual pathogens. The value of the DNA approach to emergent infections was recently illustrated by SARS (severe acute respiratory syndrome—discussed in more detail below).

Real-time PCR is starting to make a significant impact on molecular microbiology. This technique is particularly valuable because the risk for contamination is reduced (real-time PCR is conducted in a closed system—see Chapter 2, Appendix), Results from real-time PCR are quickly available. Semi-automation is possible, which is essential when dealing with common infections for which the number of referred specimens would be high. Related to real-time PCR is quantitative PCR (Q-PCR). This technique is essential for accurate viral quantitation (HIV, HCV).

Table 8.2 Some pathogens that have had their genomes sequenced, with the focus here on those likely to have major pathogenic effects[a]

Grouping	Examples
CDC category A[b]	*Yersinia pestis* (plague), *Bacillus anthracis* (anthrax)
CDC category B[c]	*Brucella melitensis* (brucellosis), *Coxiella burnetii* (Q fever), *Salmonella enterica* (typhoid fever), *Vibrio cholerae* (cholera), *Clostridium perfringens* (gas gangrene)
Others	*Borrelia burgdorferi* (Lyme disease), *Mycobacterium leprae* and *tuberculosis* (leprosy and TB), *Neisseria meningitidis* (meningitis), *Helicobacter pylori* (ulcers), *Treponema pallidum* (syphilis), various strains of *Chlamydia*, *Mycoplasma*, *Staphylococcus*, *Streptococcus*

[a] By December 2004, about 188 bacterial genomes had been sequenced. See this list as well as all other published genome projects (239 in total) on the Gold™, Genomes OnLine Database (http://www.genomesonline.org/). [b] CDC—Centers for Disease Control category A are organisms posing a major threat to national security particularly in relationship to high mortality, infectiveness and public health impact. [c] CDC category B is the next level because these organisms are easy to disseminate and cause morbidity, but not to the same degree.

Test Utility

A number of parameters are used when assessing the value of a diagnostic test. **Sensitivity** refers to the percentage of true positives that will be detected as being positive by the test—or the probability that the test will be positive when the disease is present. **Specificity** refers to the percentage of true negatives that will be detected as being negative by the test—or the probability that the test will be negative when the disease is not present (Table 8.3). The above parameters are largely a product of the test and do not alter according to the population tested. However, they do not always directly demonstrate a test's usefulness. This is better assessed by the predictive value of positive and the predictive value of negative results coming from the test.

Positive predictive value (PPV) refers to the percentage of all positive test results that are truly positive (or the probability that the disease is present when the test is positive). PPV is influenced by the specificity but also by the prevalence of what is being tested for in that population. The higher the prevalence, the higher will be the PPV since there will be less chance of false positive results. The **negative predictive value (NPV)** refers to the percentage of all negative test results that are truly negative (or the probability that the disease is absent when the test is negative). NPV is influenced by test sensitivity as well as prevalence. A high NPV will be easier to obtain if the infection has a low prevalence in a population. **Tests with high PPVs** are required for conditions in which a false diagnosis will have significant consequences; e.g., treatment regimens are potentially toxic; there are medical or psychological stigmata associated with a pos-

Table 8.3 Sensitivity and specificity in an infectious disease scenario (180 individuals in the population—50 are infected and 130 are not infected, Strohl *et al* 2001)
(See Table 2.10 for more information on this topic.)

Description of test	Number infected (from total 50) who test positive	Number not infected (from total 130) who test positive	Comments
High sensitivity High specificity	50	0	All infected people detected and no false positives recorded.
High sensitivity Low specificity	50	60	All infected people detected, but also false positives are recorded.
Low sensitivity High specificity	30	0	No false positives, but missing some infected people (i.e., getting some false negatives).
Low sensitivity Low specificity	20	70	Not all infected people are detected (i.e., some false negatives), and some detected are falsely positive.

Table 8.4 Some examples of DNA diagnostics used for infectious disease detection and monitoring (Louie *et al* 2000; Gilbert 2002)

Organism	Traditional test	DNA test	Comments
C. trachomatis	Fastidious grower; antigen detection not sensitive or specific enough.	PCR very accurate with sensitivities and specificities in the 90–100% range.	Can be detected in a number of samples and can be multiplexed as a PCR test to detect other organisms in STD, e.g., *N. gonorrhoeae*.
M. tuberculosis	Fastidious grower, but culture still plays an important role in overall management and remains the gold standard. Only way in which to measure drug sensitivity.	PCR demonstrates high sensitivity and specificity (if specimen has acid fast-staining bacteria detectable).	Particularly useful when rapid diagnosis is essential, e.g., TB meningitis.
Herpes simplex virus	Difficult to detect with conventional assays in scenarios such as encephalitis.	PCR can detect virus in CSF.	Avoids need for brain biopsy.
Bordetella pertussis	Detection through swab culture possible.	PCR-based method more rapid and easier.	Early detection possible.
HIV, HCV	Cannot quantitate with conventional assays.	PCR quantitation now available in commercial kits.	Drug treatment in AIDS can be monitored by $CD4^+$ cell counts and viral loads.
S. aureus (methicillin), *Enteroccoci* spp. (vancomycin), *M. tuberculosis* (various drugs), HSV (acyclovir), CMV (ganciclovir)	Culture for sensitivity testing (when possible) takes a few days.	PCR-based assays targeting various antibiotic or drug-resistance genes can be completed in a few hours.	As more information becomes available about the genomes of pathogens, it will be possible to test for various DNA markers to predict the pathogen's effect.

itive test as might occur in the case of sexually transmitted diseases. **Tests with high NPVs** are required when it is essential that positives are not missed, e.g., blood screening tests, treatable infections with fatal outcomes if missed.

INFECTIOUS DISEASE DNA LABORATORY

To date, DNA testing in microbiology has been directed predominantly to the detection of organisms that are difficult to culture *in vitro*, or for various reasons (delayed transport, low titre pathogens, prior treatment with antibiotics) growth is unlikely. Infections in which there is a mix of pathogens might also be usefully approached through DNA analysis. Apart from the straightforward diagnostic applications, DNA microbiological testing has been used to detect antimicrobial resistance (methicillin-resistant *S. aureus* and vancomycin-resistant *Enterococci* spp) or toxigenic forms of *E. coli*. More recently, the availability of DNA technology to quantitate HCV and HIV has been useful in planning and monitoring treatment. Table 8.4 provides some scenarios involving DNA testing.

Unlike molecular genetic (DNA) testing, commercially produced kits have made an early entry into molecular microbiology, and they allow a wider range of laboratories to participate in this technology. A very important boost to this area has been the Human Genome Project (Chapter 1). The successful completion of genomic sequencing for a number of microorganisms was both a catalyst for the human sequencing endeavour as well as the characterisation of genomes from additional model organisms. The next developments with a major impact on molecular microbiology will be microarrays (see Chapter 5, Appendix) and nanotechnology (Chapter 5). The latter will enhance the potential for point of care testing.

In contrast to the genetics DNA laboratory, the microbiology DNA laboratory faces greater technical challenges because infected samples invariably have little of the pathogen's DNA to function as a template for PCR, and the limited amount of DNA that is available will be mixed with DNA from many other organisms that are present. Because of the small target for PCR, it is not surprising that contamination from other PCR products (other samples or previous amplifications) becomes a major source of error. Another inherent problem in the microbiology setting is the potential for inhibitors in the various samples tested to interfere with DNA amplification. Finally, interpretation of some microbiological DNA results is difficult because the test shows only the presence of pathogen DNA. Whether this indicates active

infection or residual DNA after the pathogen has died cannot be determined. On the other hand, the finding of mRNA (i.e., cDNA by PCR) is more likely to indicate an active infection. If one then adds that DNA tests are relatively expensive compared to the traditional approaches, it is not surprising that many of the optimistic predictions about molecular microbiology have been slow to materialise. However, there is a steadily growing acceptance of DNA diagnostics. The threat of bioterror or emerging global infections such as was feared with SARS will best be resolved if there is a DNA or RNA component as part of the overall containment strategy.

HUMAN IMMUNODEFICIENCY VIRUS (HIV)

In December 2003, an estimated 34–46 million people were infected with HIV, and more than 20 million had died from AIDS. Included in this number are 2.1 million children under 15 years of age, and more than 2 million HIV-infected women give birth each year (http://www.who.int/whr). All regions of the world have AIDS, including Africa, Asia, Latin America, North America, Europe, the Caribbean, and Oceania. Three quarters of cases are found in sub-Saharan Africa. A very worrying trend is occurring in India, with estimates of the HIV infected somewhere between 2.2 to 7.6 million people.

There are two viral types (HIV-1, HIV-2). HIV-1 was first isolated in 1984 (Figure 8.2). A year later, a similar virus (HIV-2) was found in Paris from West African patients with AIDS but seronegative for HIV-1. HIV-2 is predominantly found in a number of West African countries, including Senegal, Ivory Coast, Gambia, Ghana and Nigeria. It has spread to other countries that have historical or other links to the African sources; i.e., France, Portugal, Angola and Mozambique. However, with the ease of air travel, sporadic cases can appear in any country. Overall, HIV-2 spreads less rapidly and takes longer to

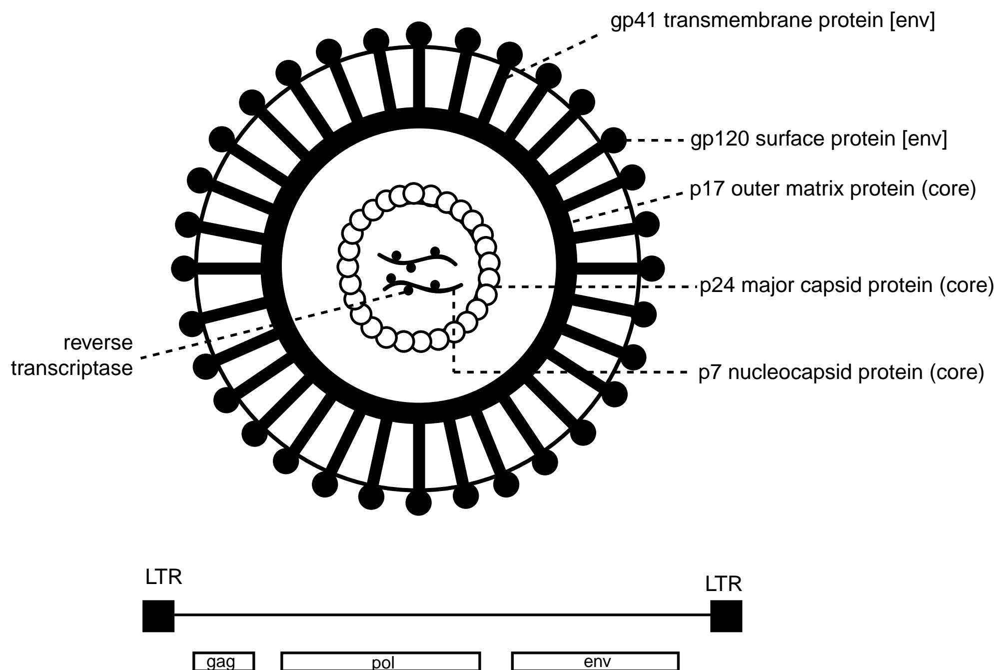

Fig. 8.2 Structure of HIV-1.
(Top) A schematic representation of the HIV genome (family Retroviridae, genus Lentivirus) shows that it comprises two identical strands of RNA within a **core** of viral proteins. Key viral enzymes are reverse transcriptase (viral DNA synthesis), integrase (integration into host cell chromosome) and protease (viral assembly). The core is surrounded by a protective envelope (env) derived from viral glycoprotein and membrane from the previous host cell. Components of the envelope are a transmembrane protein, gp41, and a surface protein, gp120. Abbreviations: gp41—glycoprotein molecular weight of 41 000; p24—protein molecular weight 24 000. **(Bottom)** A number of genes make up HIV. **Gag** (group specific antigens) codes for capsid, matrix and nucleocapsid proteins. **Pol** codes for reverse transcriptase, protease, integrase and ribonuclease. **Env** (envelope) codes for a glycoprotein precursor, gp160, that is cleaved to give envelope structural proteins gp120 and gp41. Although an RNA virus, HIV is able to produce DNA through its reverse transcriptase enzyme once it infects a cell. The viral DNA then integrates (via integrase) into double-stranded host DNA to produce the proviral form. Not shown are six regulatory genes (*rev*, *tat, nef*, *vpr*, *vpu* and *vif*) that give HIV a more complex structure compared to the animal retroviruses. LTR—long terminal repeats of the retrovirus.

develop than HIV-1. The lower viral load associated with HIV-2 may also explain why it is less likely to be transmitted from mothers to babies.

HIV-1 and HIV-2 are retroviruses (so called because they transfer the order of information from RNA to DNA) belonging to the lentivirus genus, which predominantly affects the nervous and immune systems. Transmission occurs by one of three routes: (1) sexually—both male to male and male to female, (2) blood or blood products, including HIV-contaminated needles and (3) perinatally—during birth or from breast feeding. There is presently no effective vaccine against AIDS. Perinatal transmission can be reduced by treating the mother with an antiretroviral agent, avoiding breast feeding and early weaning. Prevention is also possible through screening blood (discussed below) and the practice of safe sex. Treatment options have dramatically improved the outlook for AIDS. However, less than 10% of those requiring treatment in developing countries have access to antiretroviral drugs (Box 8.1).

The urgency and potential implications of the AIDS pandemic have resulted in many research programs directed to better diagnostic tests, more effective therapeutic regimens and a greater understanding of the viral biology. The identification of HIV viruses has required extensive changes to be made in blood screening protocols (see below). In all these areas, rDNA technology is playing a major role. In the context of the present theme of laboratory detection, molecular medicine has an important function to play since opportunistic infections are the chief cause of death in AIDS. All who have AIDS will develop infections at some time in their illness. The pathogens involved include those that normally do not produce overt disease such as *Candida albicans*, and the more exotic organisms that will not usually be seen in clinical practice, e.g., JC virus, which gives rise to a demyelinating neurological disorder called progressive multifocal leukoencephalopathy. In these circumstances, standard microbiological detection methods often prove ineffective.

Screening and DNA Testing for HIV

Many commercial ELISA (enzyme linked immunosorbent assay) kits detect antibodies to HIV-1 and HIV-2 (Figure 8.3). They are used to screen those who are at risk and blood donors. The assays are well standardised with good sensitivities and specificities. In HIV infection, screening tests must have the highest sensitivity so that positives will not be missed. False positives are then excluded by using a second assay with a high specificity, e.g., a western blot.

HIV-1 testing by DNA analysis can be directed towards the *gag*, *env* or *pol* genes of the virus (see Figure 8.2). Laboratory techniques and detection strategies (algorithms) utilising these sequences have been constructed

Box 8.1 Treatment of HIV/AIDS.

AIDS represents the clinical phase of HIV infection that by now is well established. Therefore, effective treatment must be started early and take into consideration a feature of this infection—the rapid development of mutant viruses. This occurs because (1) HIV infection is associated with significant viraemia. (2) The viral population is very heterogeneous due to an error-prone reverse transcriptase (unlike DNA polymerase, which makes few mistakes in the replication of DNA). Therefore, mutations are constantly being produced in the HIV genes, and mutant viruses will form. This, combined with the high viral titres present, facilitates the development of resistance. The key to success with AIDS treatment has been HAART (highly active antiretroviral therapy), which became available in 1996 and involves the use of multiple drugs directed to different viral pathways.

Class of antitretroviral drug	Mechanism of action	Examples
Nucleoside and nucleotide analogues	Act as DNA chain terminators, thereby inhibiting the reverse transcription of viral RNA into DNA	Zidovudine (AZT), lamivudine
Non-nucleoside reverse transcriptase inhibitors	Bind and inhibit viral reverse transcriptase	Nevirapine
Protease inhibitors	Block viral protease required for making the inner core of viral particles	Nelfinavir
Entry inhibitors	Block virions from penetrating target cells	Enfuvirtide

Combination drug therapies involve the use of three drugs having a synergistic rather than additive effect on reducing the viral load, and so they delay the development of drug resistance. Various combinations are used, for example, two nucleoside and nucleotide analogues with the third drug a non-nucleoside reverse transcriptase inhibitor or a protease inhibitor. More recently, a fourth class of drug with a completely new mechanism of action has been developed. This involves inhibiting the entry of HIV-1 into $CD4^+$ lymphocytes. The major questions now requiring resolution include when to start HAART and what are the optimal drug combinations (more than 20 drugs are approved by the FDA for treating HIV infection). Despite the use of HAART, HIV re-emerges once therapy is stopped, indicating a virostatic rather than virocidal effect. Better drugs are still needed, but more importantly, a vaccine that will allow the prevention of HIV-AIDS.

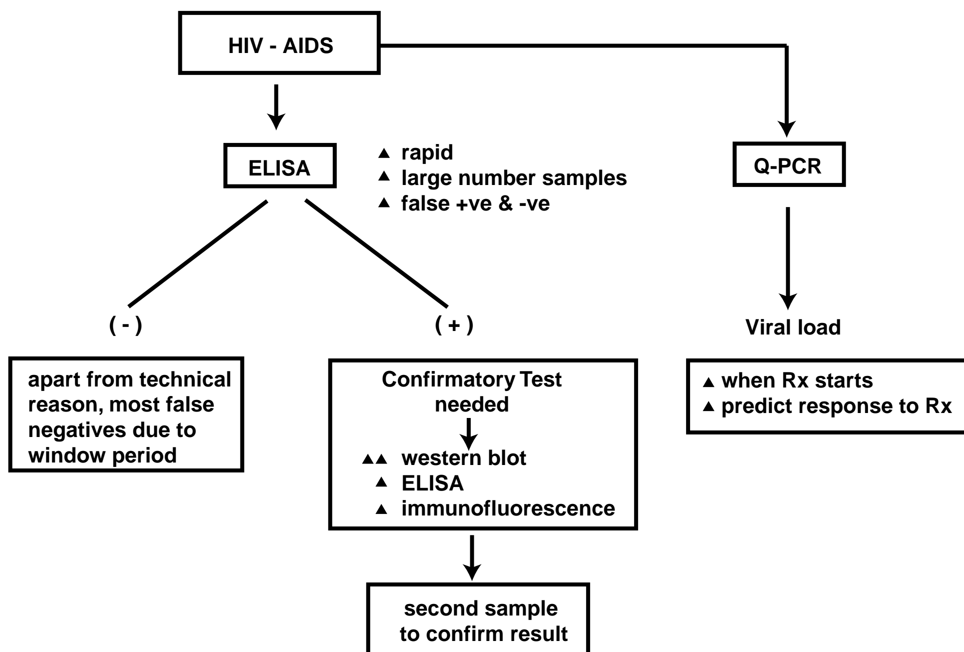

Fig. 8.3 Diagnosis of HIV/AIDS infection.
The ELISA test is the mainstay for routine HIV detection. It is rapid and able to test many samples. It has the disadvantage of both false positives and false negatives, and so confirmation by a western blot (or second ELISA or an immunofluorescence test) is essential. Apart from technical and clerical mistakes leading to a false negative result, the most common reason for this type of error is the window period since it takes between 4–12 or more weeks after infection for antibodies to form. A second independent sample is then tested to reduce the potential for technical or clerical problems before a definitive diagnosis is made. Quantitative PCR (Q-PCR) is used to assess viral load, which helps to decide when to start therapy and can be used to monitor response to therapy.

to optimise sensitivity and specificity and minimise false positive or false negative results. It has been estimated that one HIV-1 proviral copy can be detected against a background of 10^5 mononuclear cells in peripheral blood. Virus isolation is the only other method with comparable sensitivity although various problems with this test exclude it as a routine assay.

Variations of the PCR technique including nested PCR (see Chapter 2, Appendix) can be used to reduce the false positive rate, which is due, in most cases, to contamination of samples or reagents. On the other hand, false negative results can reflect the presence of inhibitors in the specimen being tested or chemical substances such as anticoagulants. Another source of a false negative result with HIV occurs because of the considerable diversity found in the viral DNA sequence so that a mismatch between the usual primer pairs inhibits the subsequent PCR step. This difficulty can be avoided by use of primer pair combinations derived from conserved segments of the viral genome. For the present, serological testing for HIV-1 and HIV-2 remains the preferred screening method for at-risk individuals, including blood donors, because it is fast, relatively cheap and able to be automated (see also blood screening below).

TRANSFUSION-RELATED INFECTIONS

A number of the important recent viruses have reached notoriety through their association with blood transfusions. Previously, blood transfusion services based their donor and blood screening programs on detecting antibodies or antigens in the donor or blood supply. However, this approach has been shown to be inadequate, and an important new addition to the screening protocols is the use of PCR to identify viral DNA or RNA. The advantages of a NAT assay include (1) higher sensitivity and (2) greater reliability during the window period, which is the time between a blood donor becoming infectious and donor screening tests becoming positive; i.e., seroconversion has occurred.

PCR-based assays for screening blood donations are used to test (1) pools of donations, for example, 16–24 donations simultaneously, and (2) individual donations. The former is more rapid and cheaper, but the very rare case of HIV and hepatitis C virus (HCV) being missed means the testing of individual donations is the method of choice. Screening by NAT has a number of potential drawbacks, including (1) It involves more sophisticated technology (therefore, trained staff are needed, and tests

Table 8.5 Some success stories with screening in blood transfusion services (Goodnough 2003)

Virus	Screening	Outcomes[a]
HIV	Donor and blood with full-range tests including NAT. First blood-related infections in 1982.	In the USA, there were 714 HIV cases in the year before testing was implemented. That number fell to about five cases per year in the next five years after NAT testing started in 1999. Risk of blood transfusion–acquired HIV in the post-NAT era is 1 : 1 900 000.
HCV	Donor and blood with full-range tests including NAT.	There are serious consequences of blood-borne HCV infection. However, following testing protocols, HCV is a rare complication. Risk of blood transfusion–acquired HCV in the post-NAT era is 1 : 1 600 000.
HBV	Donor and blood with third-generation serologic assays for hepatitis B surface antigen.	Risk of blood transfusion–acquired HBV is still relatively high (1 in 220 000) although only about 10% of post-transfusion hepatitis is due to HBV. Unlike HCV, risk of chronic liver disease is less with HBV.
CJD, vCJD	No useful test available.	In the USA, deferral protocols have been in place since 2000; i.e., individuals living in certain European countries during defined times are excluded. No direct evidence that blood can transmit CJD or vCJD, although the infection is transmitted with tissues such as cornea and pituitary extracts. In the UK, plasma for fractionation is obtained from the USA.
West Nile	Most useful is NAT for viral RNA. Serologic testing of donors is not sensitive.	In 2003, 23 cases were reported—resulting in the development in a very short time (2003) of a PCR-based test used to screen all donors. Also included in the donor questionnaire is history of fever with headaches in the week before donation (yes answer leads to deferral).

[a] It is worthwhile comparing infectious risks of blood transfusions with incompatible blood transfusions with their associated 10% mortality. The FDA reports over twice the frequency for incompatible blood transfusion compared to all infections, and in the UK, adverse events related to incompatible blood transfusions were 10 times higher than for infectious diseases.

are invariably more expensive). (2) Assays are technically demanding, including the strict requirement to prevent contamination. (3) Results from NATs take longer than those from serologic ELISAs (enzyme linked immunosorbent assays). However, in response to a number of cases of contaminated blood, many blood transfusion services have opted for NAT testing.

Blood transfusion services screen blood and donors for a range of infectious agents, e.g., hepatitis B virus (HBV), HCV, HIV-1, HIV-2 (and in some regions human T-cell lymphotropic virus types I and II [HTLV-I, HTLV-II]), syphilis, cytomegalovirus (CMV) and, more recently, West Nile Virus in the USA. Some screening tests are undertaken only in selected circumstances; e.g., CMV-free blood is required for an immunosuppressed patient, fetus or neonate.

The risks of blood-borne viral infections have dramatically decreased with the implementation of donor questionnaires that allow self-exclusion (particularly important for infections that are not routinely tested for), donor and blood testing with ELISA and PCR-based techniques. The latter's sensitivity is several magnitudes higher than culture or ELISA methods. To exclude the potential for slow virus transmission (i.e., CJD and vCJD) through blood, transfusion services have deferred the use of donors who have lived in the UK over certain time periods (Table 8.5). Other reasons for deferral include fever with headaches the week before donation (possibility of West Nile Virus and others) or travel to risk regions (for example, the possibility of malaria).

Apart from HIV, another success with blood transfusion screening has been HCV. Since the single-stranded ~10 kb RNA sequence of the HCV was reported in 1989, it has been possible to use an enzyme immunoassay to detect antibodies against a number of viral antigens in patient testing or blood donor screening. However, the first-generation immunoassays gave rise to many false positives. This problem with serological testing was overcome by the availability of more sophisticated second- and third-generation enzyme immunoassays. Similar to what happens with HIV, the resolution of false positive results is undertaken by an additional assay such as an immunoblot that incorporates multiple viral-specific antigens. Viral antigens in many of the modern tests have been prepared by using rDNA expression systems such as *E. coli* or yeast (see Chapter 6). Indeterminate or equivocal results can be further evaluated with PCR.

PATHOGENESIS

The pathogenesis of many infections, particularly viral ones, has been deduced from experimental strategies based on light and electron microscopy, cell culture and immunoassay. To these research tools can now be added nucleic acid (DNA, RNA) probes for *in situ* hybridisation or NAT. Advantages provided by DNA techniques include the ability to detect latent (non-replicating) viruses and to localise their genomes to nuclear or cytoplasmic regions within cells. Tissue integrity remains preserved during *in situ* hybridisation, and so histological evaluation can also be undertaken. Nucleic acid probe techniques or NAT can be manipulated to enable a broad spectrum of serotypes to be detectable. This is particularly valuable in those emerging infections where the underlying serotypes are unknown. More recently, the most powerful application of DNA techniques is the ability to sequence whole genomes, and so identify the pathogen, and from its genomic sequence (1) predict its role in disease pathogenesis and (2) identify regions of the genome suitable for NAT-based detection methods.

HOST RESISTANCE

The host's response to an infection involves a complex mix of genetic and environmental factors. In humans, evidence for a genetic component contributing to the outcome of an infectious disease comes from the observation that some ethnic groups are more resistant to infections, whereas others appear to have an increased susceptibility; e.g., resistance to malaria in black Africans and susceptibility to chronic carrier state for HBV in Chinese. Not all exposed to HIV-1 get infected, and those who do progress to AIDS show different responses. For example, there can be a rapid progression within 1 to 5 years, or individuals can demonstrate a more benign progression extending up to 20 years. Because host resistance is likely to represent multiple genetic effects—i.e., QTLs (quantitative trait loci), which interact with the environment—they are difficult to detect and so have been sought by the usual molecular genetic approaches, particularly association (case control) studies and the investigation of candidate genes (see Chapter 4 for further discussion of this strategy and QTLs).

HIV-1 Infection

HIV-1 targets the macrophages, monocytes and T lymphocytes that carry the CD4 cell surface protein. HIV-1 first attaches to CD4 and then the chemokine receptor CCR5. Once this occurs, the HIV gp41 protein can penetrate the cell membrane, enabling HIV to infect the cell. In some circumstances, particularly as the disease progresses, a mutation in the *env* gene enables a move away from the CCR5 receptor to the CXCR4 receptor. Therefore, two host-related genetic factors can influence the infectivity of HIV-1 in humans. They are (1) chemokine receptor genes and (2) major histocompatibility complex (MHC).

CCR5 is a chemokine and functions as a co-receptor required for HIV to enter cells. CCR5 is highly polymorphic, with variants found in different ethnic groups. One important variant involves a 32 bp deletion in the coding region leading to a truncated protein. It is now known that homozygotes for CCR5 Δ32 (approximately 1% of Caucasians) have significant resistance to HIV infection because the virus has lost one of its entry points into the cell. Heterozygotes do not appear to have enhanced resistance to HIV infection although progression to clinical AIDS is delayed, and viral loads are lower. Other genetic variants related to CCR5 have been reported to influence HIV infection and progression (Table 8.6).

The second and probably the more important of the genetic factors influencing HIV infections came from an observation of apparently HIV-resistant female sex

Table 8.6 Genetic variants in chemokine receptors influencing HIV infectivity (O'Brien and Nelson 2004)[a,b]

Variant	Mechanism
CCR5	Δ32 defect decreasing the amount of this receptor. Homozygotes for this have a strong resistance to HIV infection (although this is not absolute). Progression to AIDS is slowed in heterozygotes.
CCR5	Apart from the 32 bp defect, mutations in the promoter have also been associated with increased gene function, thereby accelerating the development of AIDS.
RANTES (*CCR5* ligand)	Mutations in the promoter can affect both susceptibility as well as HIV progression, depending on how RANTES expression is affected.
CCR2	The variant V64I appears to delay progression of HIV perhaps by impairing the transition from the CCR5 receptor to an alternative one (CXCR4).
CXCL12	Variant in the 3′ end appears to impair HIV entry into the cell perhaps through the CCR5 to CXCR4 transition.

[a] Unlike the protective effect of the CCR5 Δ32 defect, there is some controversy about the role played by the other genetic variations because they have all been identified through genetic association studies. [b] Two other important genes that influence progression from HIV to AIDS are *IL10* and *IFNγ*. These genes produce cytokines that inhibit HIV replication. Hence, genetic variants that lead to overexpression of these genes will favour a milder disease, and the converse occurs with genetic variants that are associated with reduced expression.

Table 8.7 The role of the MHC in controlling infections[a]

HLA class	Cells expressing this class	Peptides recognised	Immune response generated
Class I (HLA A, B, C)	All nucleated cells	Those derived from intracellular pathogens, e.g., viruses	$CD8^+$ T cells initiate cytotoxic cell response.
Class II (HLA DP, DQ, DR)	Expressed only on antigen-presenting cells, i.e., macrophages, dendritic cells and B lymphocytes	From extracellular and intravesicular pathogens, e.g., bacteria, fungi and some phases of viral infections	$CD4^+$ T cells lead to cytokine production and promote B cell antibody production.

[a] The MHC (in humans, it is usually called HLA) locus codes for cell surface proteins are important for immune surveillance since T cells do not respond to foreign peptides from a pathogen unless they are coated (or presented) with MHC proteins.

Table 8.8 Association between HLA type and susceptibility to infection (Carrington and O'Brien 2003)

Infection	HLA type	Mechanism
HIV	Homozygosity for any type	Faster progression of HIV because a narrower range of HIV peptides is presented for cytotoxic T cell response.
HIV	B27	Slower progression of HIV appearing to reflect a super efficient binding of p24 gag HIV epitope by those positive for HLA B27.
HIV	B57	Slower progression of HIV perhaps because B57 molecules target a wider range of HIV peptides involving gag and reverse transcriptase.
HIV	B35	Faster progression of HIV secondary to failure of B35 alleles to bind HIV peptides due to single amino acid change. Carriers have a more rapid progression, while homozygous individuals develop AIDS within half the median time it takes those who are B35 negative.
Malaria	B53	Protection from severe infection in West Africa.
Myco bacteria	DR2	Increased susceptibility documented in some populations (Asia, India) but not others.
HBV	DRB1*1301 and 1302 (subtypes of HLA DR13)	Protection against chronic HBV through enhanced viral clearance.
HCV	DQB1*0301	Enhanced clearance of HCV (Europeans and American blacks); however, in American whites associated with viral persistence.
	DRB1*0101	Contradictory results in relation to association with self-limiting HCV infection or enhanced clearance.

workers in Nairobi. These women were shown to have a particular HLA type (HLA A2, HLA A28), and it has been proposed that this enhanced their resistance through a better presentation of HIV antigens to T lymphocytes. Therefore, the virus was more rapidly cleared by the immune system. Subsequently, there have been many associations described between HLA types and increased resistance or greater susceptibility to HIV infection and its progression to AIDS (Table 8.7).

Another observation involving HLA is that mothers and children who are mismatched at the MHC are less likely to have perinatal transmission because immune surveillance is enhanced. However, the HLA protective effect is not observed if HIV is acquired through breast feeding. Other HLA associations in AIDS (as well as other infections) have been reported, including polymorphisms of interleukins IL4 and IL10 (Table 8.8). Like association studies in genetic disorders, the reports have produced many correlations between genetic markers and response to infections although they often remain controversial. The confusion that can be found with association studies is well demonstrated by the HCV example in Table 8.8.

Malaria

Each year there are more than 500 million cases of falciparum malaria in Africa, and around 1–2 million deaths will result. A resurgence in malaria reflects socio-economic factors (poverty, overcrowding), emergence of mosquitoes that are resistant to insecticides and development of drug resistance. The two common forms of malaria (*P. falciparum* and *P. vivax*) produce severe anaemia, and in addition, *P. falciparum* is associated with cerebral malaria, respiratory and metabolic complications. This differing spectrum is partly explained by *P. falciparum* being able to invade a large proportion of red

blood cells compared to *P. vivax,* which invades only the reticulocytes. Another explanation is the mode of entry of these parasites into red blood cells, with *P. falciparum* having a number of different invasion pathways compared to *P. vivax*, which is able to enter only red blood cells that carry the Duffy blood group.

Host factors providing some protection from malaria have been identified. They include single gene effects seen in haemoglobinopathies such as sickle cell anaemia or thalassaemia, and the Duffy negative blood group. The former are explained by suboptimal red blood cell environments that do not provide a good milieu for the parasite. The Duffy negative blood group has a more powerful protective effect since it is the receptor by which *P. vivax* enters the red blood cell. Thus, *P. vivax* is not seen in West Africa because the populations are Duffy negative.

Hepatitis B

Although vaccination for HBV has made a very significant reduction in new cases, worldwide there still remain about 350–500 million infected individuals. Long-term complications with this DNA virus occur in about 15% of those infected and include chronic hepatitis, cirrhosis and hepatocellular carcinoma. Why some go on to develop a chronic carrier state is not known, with the course of the illness after infection very variable. This has been explained on the basis that it involves the viral genotype and the host's immune responses; i.e., following acute infection, a vigorous T cell response against the various viral-specific components (core, envelope and polymerase) limits the infection. In contrast, those with a poor cellular immune response are more likely to develop a chronic carrier state. In this scenario, the MHC will play a key role since class II molecules from the HLA complex are critical for T cells to function. Evidence for an MHC-related effect in hepatitis B infection is presented in Table 8.8.

Animal Models

Polygenic traits contributing to pathogen susceptibility or resistance in humans would be difficult to separate into their individual components because of the genetic variability in the human genome. However, this can be overcome by using different strains of inbred animals, particularly mice. Since chromosomal segments in the mouse can be traced to their homologous or syntenic regions in humans, it becomes possible to identify a genetic locus or gene in a mouse and then go back to the corresponding region in the human genome to find the equivalent gene.

Forward genetic screens (phenotype to genotype) result from the observation that a particular mouse strain (or one created by, for example, a chemical mutagen such as ENU—Chapter 5) has a phenotype that alters the animal's expected response to an infection. An example would be a mouse strain shown to have resistance to *Leishmania donovani* infection. From this observation, a gene was isolated by conventional positional cloning. The gene was called *Nramp1* (now renamed *Slc11a1*). Mice with mutations in this gene (particularly G169D) are resistant to three intracellular pathogens (*Mycobacterium bovis*, *Salmonella typhimurium* and *Leishmania donovani*). This gene is likely to play a key role in infection since it is exclusively expressed in macrophages, key cells in the control of the three intracellular pathogens just mentioned.

A reverse genetic screen (genotype to phenotype) is exemplified by the transgenic knockout mouse when the particular phenotype associated with a gene can be confirmed by inactivating that gene. One interesting knockout involves the removal of the TNFα (tumour necrosis factor) gene, which leads to a mouse that is very susceptible to the pathogens *M. tuberculosis* and *L. monocytogenes*. A similar susceptibility is seen if the mouse's interferon-γ gene is knocked out. Both these genes play key roles in the immune response.

Another strategy allowing a mouse model to be used for an infection that is found exclusively in humans is to "humanise" the mouse for the particular host-tropism that explains the species specificity. An example of this is the polio virus that does not normally infect mice. However, transgenic mice that express the human poliovirus receptor CD155 develop polio if infected intracerebrally, but not if they are infected orally. In addition to creating a suitable animal model, this experiment also indicates that the oral phase of infection with poliovirus is likely to involve another receptor.

DRUG RESISTANCE

Antimicrobial Drugs

Resistance to antibiotics has always been a concern. However, new antibiotics in the 1950s–60s temporarily addressed the problem, and this false sense of security was reinforced in the next two decades with newer and more powerful antibiotics. Today, the increasing trend in antibiotic resistance is alarming, and is not being matched by new drug development. This led to the World Health Organization in 2001 developing a plan to address what has become a global problem.

Resistance to antibiotics occurs by three major mechanisms: (1) reduced antibiotic uptake by a cell or increased efflux from the cell, (2) modification or inactivation of the antibiotic and (3) altering the target for the antibiotic. The development of resistance may be an intrinsic feature of an organism, or it may result from mutations or the acquisition of resistance genes. Transfer of resistance genes can occur via plasmids or trans-

posons. The latter represent mobile DNA elements that can move between plasmids or between plasmids and chromosomes, thus having the potential to disseminate widely (and stably) drug resistance genes.

The genes involved in antibiotic resistance are being characterised, enabling a better understanding of how this develops and how to detect it quickly by DNA-based approaches, and finally, identifying strategies by which the resistance pathways can be bypassed. Apart from the inappropriate use of antibiotics in medicine, agriculture and animal feeds (about 50% of antibiotics are used in the beef and poultry industry!) contributing to the increasing resistance, other factors involved include travel and tourism.

Pneumococci are important pathogens in the community leading to central nervous system infections, pneumonia and otitis media in children. Resistance to macrolide antibiotics (e.g., erythromycin) in some countries is reaching alarming proportions; for example, 65% of *Streptococcus pneumoniae* are resistant to erythromycin in parts of Asia. Two major mechanisms explain resistance in this organism: (1) Bacteria carrying the *ermB* gene can methylate a specific amino acid on the ribosome (the site of action for macrolides) and so interfere with binding of the antibiotic to this region. (2) Bacteria carrying the *mefA* gene (an efflux pump gene) can export out the antibiotic and so protect the ribosome.

Multidrug resistance (MDR) occurs with both antibiotics and anti-cancer agents, and refers to the development of resistance to a range of drugs that may be unrelated or differ widely in their structure or target. Common pathways are seen with MDR. One of these involves the P-glycoprotein (*MDR1*) gene, which is a plasma membrane–spanning multidrug transporter protein. One of the most important mechanisms for MDR is the ability to extrude out the antibiotic (or anti-cancer drug) from the cell (Figure 8.4). Antibiotic resistance to many gram negative bacteria (*Pseudomonas aeruginosa*, *Acinetobacter* spp, and the Enterobacteriaceae) occurs through an efflux-mediated mechanism.

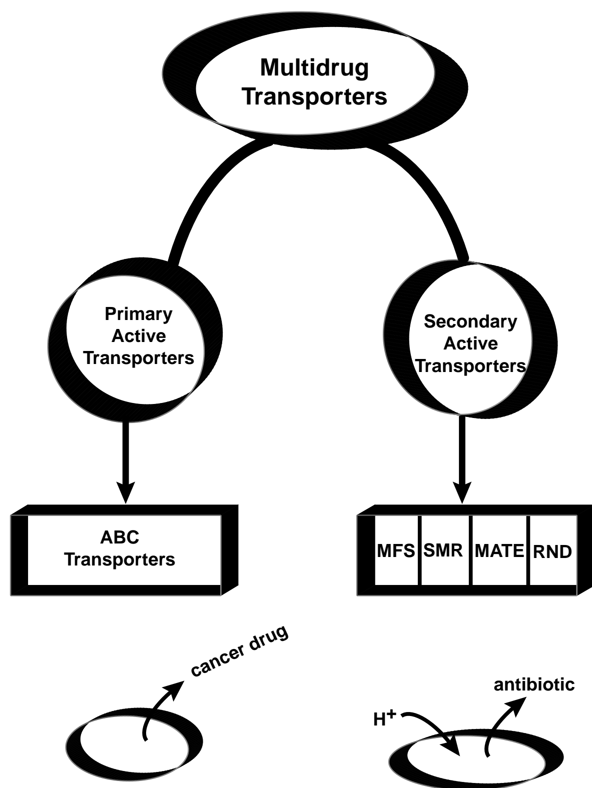

Fig. 8.4 Drug resistance pathways involving multidrug transporters.
Two major classes can be described. The first is the ABC (ATP Binding Cassette) transporters that utilise ATP to transport drugs out of the cell. This class is mostly found in eukaryotic cells and would be predominantly involved in resistance developing to cancer drugs, although it is also implicated in drug resistance for a wide range of pathogens. The second includes a number of families (MFS, SMR, MATE and RND). They move drugs out of the cell at the same time as they move in protons or sodium. Members of this class are found predominately in prokaryotes and would be important in antibiotic resistance.

Mycobacterial Infection

Rifampicin occupies a pivotal place in the World Health Organization's multidrug resistance program for tuberculosis (TB) and leprosy. Despite this, about 3.2% of new TB cases are caused by multidrug-resistant strains. The development of resistance has major therapeutic and public health implications. Since *M. leprae* grows slowly, it is essential to confirm the development of drug resistance by alternative means to the traditional culture approach. Similarly, to avoid the use of what will be ineffective drugs (a cost consideration as well as a way to optimise antimicrobial therapy), the Centers for Disease Control recommends that resistance patterns for TB should be reported within 28 days of the specimen being received. However, this is a challenge because of the slow-growing *M. tuberculosis*. Therefore, an alternative approach is to use molecular diagnostic techniques to identify resistance genes in the mycobacteria.

Until recently, the use of various drug combinations selected from isoniazid, rifampicin, ethambutol, streptomycin and pyrazinamide as well as improved living standards was instrumental in producing a declining incidence for tuberculosis. However, this is now changing. The number of new cases is increasing. The traditional dogma that active cases usually arise from infections acquired years earlier is no longer applicable since DNA studies have shown that approximately one third of active cases in some cities are the result of person-to-person transmission. Contributing factors to

Table 8.9 Drug resistance to mycobacterial infection (de Viedma 2003)[a]

Drug	Mechanism for resistance
Rifampicin	Involves the *rpoB* gene encoding the β subunit of the DNA-dependent RNA polymerase. Mutations in this gene are found in a specific 81 bp region.
Isoniazid	Involves at least four different genetic loci: *katG*, *inhA*, *ahpC* and *oxyR* genes, which affect different metabolic pathways. One particular mutation (*katG*315) has a frequent association with high-level resistance.
Ethambutol	Mutations are found in the *emb*CAB gene involved in metabolic pathways.
Streptomycin	Mutations are found in *rrs* (coding for 16S RNA) or *rps*L (codes for the ribosomal protein 12S).

[a] Despite the many resistance-related mutations being identified, some resistant strains of *M. tuberculosis* do not have mutations in these genes.

Table 8.10 Drug resistance to malaria (Le Bras and Durand 2003)

Drug	Mechanism for resistance
Chloroquine	Impaired uptake of chloroquine by the parasite's digestive vacuole due to mutations in the *Pfcrt* gene is responsible for resistance to this drug. Mutations in genes *Pfmdr1* and *Pfcg2* may also be important.
Antifolates (pyrimethamine and proguanil)	Mutations in *Pfdhfr* gene (dihydrofolate reductase) which is the target of the antifolates. Point mutations interfere with inactivation of the dihydrofolate reductase.
Sulfonamides and sulfones	Mutations in *Pfdhps* gene (dihydropteroate synthase) which is the target of these drugs. Mutations interfere with inactivation of dihydropteroate synthase.
Sulfadoxine-pyrimethamine	Through selection of *dhfr* and *dhps* mutants.
Amino alcohols (quinine, mefloquine and halofantrine)	Unknown.

the increase in tuberculosis and the finding of multidrug-resistant strains are HIV infection, intravenous drug use and the decline in living standards resulting from political changes or war. At the molecular level, the genes associated with drug resistance are being defined (Table 8.9). To deal with these challenges, modern global strategies directed to tuberculosis (and leprosy) will require laboratory facilities that utilise state-of-the-art technology to provide health professionals with accurate information in an efficient and timely fashion.

Malaria

During the latter half of the twentieth century, chloroquine was the drug of choice for treating falciparum malaria. However, the spread of drug-resistant *Plasmodium falciparum,* and to a lesser extent *Plasmodium vivax*, has made the prophylaxis and treatment of malaria a global public health issue. Chloroquine-resistant *P. falciparum* is present in every major region where malaria is endemic. Air travel means that drug-resistant strains can appear in any city. Resistance is now spreading since the 1950s from a limited number of foci in South East Asia and South America. The epidemiology is consistent with multigenic effects, i.e., rare events occurring initially in South East Asia or South America and then spreading slowly worldwide. In contrast, resistance to pyrimethamine and proguanil has arisen independently in many different regions where these drugs have been used, consistent with a single gene being involved.

The molecular basis for resistance in malaria is slowly being unravelled, and many genes are now implicated (Table 8.10). As the molecular defects become more comprehensively characterised, it will be possible to predict the drug resistance pattern for various geographic regions and so optimise drug selection. It might also be feasible to use other drugs or genetic-based therapies to bypass the resistance defect in the parasites, allowing the continuing use of what are cost-effective and relatively safe drugs such as chloroquine. Another positive development in the therapy of malaria is the availability of the DNA sequence from the genomes of the important human and mouse malaria parasites. This development will enable new targets to be found, i.e., genes for metabolic pathways that differ between the parasite and humans.

HIV

Monitoring response to AIDS drug treatment can be undertaken by measuring the CD4$^+$ cell count, as well as the assessment of viral load by Q-PCR. In the USA, about half the patients are infected with viruses that have some resistance to drugs. The mutations causing resistance, as well as their likely modes of action, are well understood for HIV. They include many single base changes and the occasional insertions of a few amino acids. Cross resistance, once it occurs, is restricted to drugs within the same class although all classes of drugs can develop resistance, which tends to occur slowly over a period of time (Box 8.1 describes the various drugs). Resistance is the result of many mutations accumulating within HIV, although one exception is the M184V mutation in reverse transcriptase, which alone produces complete resistance to lamivudine. When plasma viral loads rise despite therapy, a change in regimen is needed. Because of the

cross-resistance within classes, what drug to substitute is not an easy decision. Assays to detect HIV resistance include genotypic (detecting RNA mutations) as well as phenotypic (*in vitro* viral cultures).

VIRULENCE FACTORS

Streptococcal Virulence Factors

Group A streptococcus (GAS) is responsible for a wide range of human infections. Virulence factors in this organism produce a broad spectrum of toxic effects from antiphagocytic, cellular adherence, internalisation, invasion and systemic toxicity. An important toxin associated with GAS is now better understood through analysis of its gene. This is the β haemolysin effect, which was difficult to study without DNA technology. The β haemolysin effect in GAS is due to a powerful cytotoxin called streptolysin S, which has a very broad toxic effect on cells and is non-immunogenic. The gene for streptolysin S has now been isolated and is called *sag*. It comprises 9 different contiguous genes *sagA* through to *sagI*. The corresponding gene for β haemolysin in group B streptococci is *cyl* and involves 11 contiguous genes.

Helicobacter Pylori *Virulence Factors*

H. pylori is an important gram negative slow-growing bacterium that colonises the gastric epithelium and can lead to chronic gastritis, ulceration and cancer. Infection is strongly associated with socioeconomic status, with prevalences >80% in developing countries and much lower ones (20–50%) in affluent regions. Disease outcome depends on a number of factors including bacterial genotype. Infection with *H. pylori* can be treated with antibiotics. However, because it is so common in some communities, a vaccine approach for prevention would be more economical and effective. To identify potential targets for vaccines in this organism, the various virulence factors have been identified. They include urease, vacuolating toxin, a cytotoxin and an adherence factor. The genome of this bacterium was sequenced in 1997, and from this it was possible to understand better the bacterium's acid tolerance and genes involved in pathogenesis. A number of the virulence-producing genes have been characterised. They include *vacA*—exotoxin-producing vacuoles in epithelial cells associated with greater risk for ulcers; *cag* PAI region associated with the induction of a more intense host inflammatory response leading to more severe disease in Western populations. The significance of *cag* PAI in the developing world is less obvious because almost all strains are *cag* positive.

CANCER

DNA and RNA viruses cause tumours in humans. Oncogenic viruses include hepatitis B (DNA), hepatitis C (RNA)—hepatocellular carcinoma, and papillomavirus (DNA)—cervical cancer. The Epstein-Barr virus (EBV), a DNA virus, is associated with the development of both lymphoid and epithelial tumours.

Hepatitis B Virus (HBV)

The hepatitis B virus is a hepatotropic DNA virus. Its replication cycle within the liver nucleus leads to the formation of mature virions via reverse transcriptase, and they are exported from the cell. Alternatively, the newly formed viral DNA can be recycled back into the nucleus for conversion to a plasmid form that generates further genomic transcripts and so maintains a constant intranuclear pool of templates for transcription. The HBV replication cycle within the hepatocyte is not directly responsible for liver damage, consistent with the observation that many HBV carriers are asymptomatic with minimal liver damage. It has also been observed that those with immune defects tend to be chronic carriers of HBV but usually in association with mild liver damage. Therefore, it has been proposed that liver damage following chronic HBV infection is predominantly the result of the host's immune response, in particular: (1) cytotoxic T cells, natural killer T cells and (2) inflammatory products, including tumour necrosis factor (TNF), free radicals and proteases.

Most primary HBV infections in adults are self-limited (compare this with hepatitis C virus discussed under Future). About 5% of primary infections in adults continue and lead to persistent infection. It is estimated that about 350 million people worldwide are HBV carriers. About 20% of chronic carriers go on to develop the serious complication of cirrhosis. Liver transplantation in HBV will be associated with a recurrence of the infection in more than 80% of patients unless preventative measures are undertaken to reduce the risk for reinfection. They include the use of hepatitis B immune globulin and an antiviral agent such as lamivudine that is also used for treating AIDS (see Box 8.1). Another serious consequence of chronic HBV infection is hepatocellular carcinoma, with carriers being 100 times more likely to develop this than non-carriers. Those who are HBeAg positive carriers are particularly at risk, which is not surprising since this serologic marker is associated with high HBV titres. Despite the HBV genome being sequenced and characterised, it is disappointing that even today there is incomplete understanding how this DNA virus leads to the development of hepatocellular carcinoma.

Papillomavirus

Papillomaviruses are DNA viruses that exhibit species specificity and induce hyperplastic epithelial lesions as a result of infection. Cervical cancer is the second most common cancer in women worldwide, and there is now unequivocal evidence implicating certain types of human papillomavirus (HPV) as the primary cause of cervical cancer and its precursor, cervical intraepithelial neoplasia. HPV is acquired mainly through sexual activity. More than 80 types of HPV have been identified, and about 40 can infect the genital tract.

Cervical cancer begins as a preinvasive neoplastic change in cells (histologically described as CIN—cervical intraepithelial neoplasia). This may regress, remain unchanged or progress to an invasive malignancy. Human papillomavirus has been implicated in the various stages of cervical cancer with different types correlating with the histological findings. Human papillomavirus has more recently been implicated in anal cancer (particularly in those who are HIV positive), penile, laryngeal and oral cancers. Human papillomavirus–related skin cancers can be found in the immunosuppressed.

Human papillomaviruses induce cellular transformation and may also interact with other viruses, oncogenes or carcinogens to bring about neoplastic changes. The virus is not the sole factor in the progression to invasive cervical cancer (Figure 8.5). The E6 and E7 oncoproteins code for proteins essential for viral replication in HPV types 16 and 18. E6 binds to and inactivates *TP53*, thereby disrupting a key cell cycle checkpoint (see Figure 4.13). The E7 oncoprotein binds to and inactivates retinoblastoma gene products, leading to further uncontrolled cell cycle progression.

The various types of human papillomaviruses can be distinguished on the basis of their DNA sequences. Comparisons between histological findings and viral types have enabled classification into three groups—low, intermediate and high risk (Table 8.11). Commercial kits based on PCR assays are now available to identify most of the high-risk HPVs. This test is considered to be very sensitive. However, as mentioned previously, a DNA test indicates only the presence of viral DNA. It cannot predict whether the infection is active or transient. Hence, the suggestion that HPV DNA testing would replace the traditional Papanicolaou smear is incorrect, particularly in young women who are more likely to have a transient HPV infection. However, used in conjunction with the Papanicolaou smear, the papillomavirus DNA screen will be helpful if there is a suspicious result, or this combination might become the preferred method for screening women over 30 years of age. In this way the combined tests will increase the overall sensitivity of screening, and so increase the interval between testing.

Epstein-Barr Virus (EBV)

EBV is a ubiquitous human herpes virus that infects most adults. After being infected, the individual remains an EBV carrier for life. In developing countries, primary EBV infection is asymptomatic and occurs early in life. In developed countries, the primary infection is delayed until adolescence or adult life, when it manifests as infectious mononucleosis. An *in vitro* characteristic of EBV is its ability to immortalise lymphocytes, an application that

Table 8.11 Classification of HPV types and their risk for cervical cancer (Munoz *et al* 2003)

High-risk HPV (probable high risk)	Indeterminate-risk HPV	Low-risk HPV
Types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82, (26, 53, 66)	Types 34, 57, 83	Types 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, CP6108

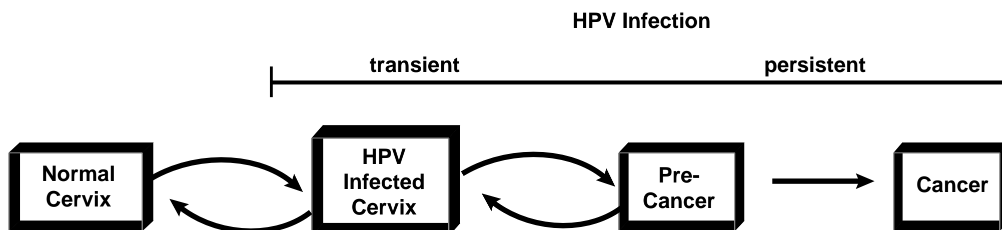

Fig. 8.5 Human papillomavirus (HPV) in cervical infection.
Similar to what has been described for colon cancer, the development of cervical cancer is a staged process predominantly in response to infection with HPV. Infection can be transient (and so reversal of the HPV effects on cervical epithelium is possible), but once HPV infection is persistent, it can lead to pre-cancerous lesions that can progress to cervical cancer. Other factors associated with the development of cervical cancer include early sexual activity (<16 years of age), >4 sexual partners, and a history of genital warts. Other independent risk factors that might interact with the HPV include HIV infection, immunosuppression and cigarette smoking.

is very useful in the research laboratory to provide a permanent supply of a particular cell line (or an unlimited source of DNA). In the immortalised lymphocyte, EBV does not replicate but remains as multiple extrachromosomal copies of the circular viral DNA genome in the cell. Latent infection with EBV is accompanied by the expression of a set of viral latent genes. These genes and their respective proteins are well characterised and include (1) six nuclear antigens: EBNAs 1, 2, 3A, 3B, 3C and LP; (2) three latent membrane proteins: LMPs 1, 2A and 2B and (3) EBERs 1, 2—small, non-polyadenylated non-coding RNAs.

Inappropriate expression of EBV latent genes leads to tumour development. Tumours in which EBV is found include Burkitt's lymphoma, post-transplant B cell lymphoma, Hodgkin's disease and nasopharyngeal carcinoma. In each, various latent genes and proteins will be detected. Two important ones (these are always present in the laboratory-transformed lymphoblastoid cell lines) are EBNA2 and LMP1. The former is a transcriptional regulator; hence, inappropriate expression of this gene will have far-reaching effects on cell proliferation. LMP1 in experimental studies behaves as an oncogene. Nasopharyngeal carcinoma is a tumour that is predominantly found in China and South East Asia. The latency genes EBNA1, EBERs and LMP1 are frequently found in this tumour. A recent study used Q-PCR to quantitate EBV-specific DNA (in the form of EBNA3 and LMP1) in the plasma and tumours from nearly 100 patients with nasopharyngeal carcinoma. This study showed that the amount of EBV DNA in the plasma correlated closely with the stage of the cancer with the most DNA found in tumours that had metastasised. The authors (Lin *et al* 2004) suggested that monitoring for EBV DNA in the plasma was an effective way to predict outcome to treatment.

EPIDEMIOLOGY

Conventional typing of pathogens based on their phenotypes (phage susceptibility; biochemistry, antigen profiles; antibiotic resistance; immune response; fimbriation; etc.) is not always successful in epidemiological studies. A changing spectrum of infectious agents, particularly in the immunocompromised host and in hospital outbreaks, has meant that newer epidemiological approaches are required to complement or replace the more traditional methods. Five strategies based on characterisation of pathogen-derived DNA or RNA are useful in these circumstances: (1) nucleic acid hybridisation, (2) plasmid identification, (3) chromosomal DNA banding patterns, (4) PCR and (5) sequencing.

ANTIGENIC VARIATION

Influenza A

The three RNA influenza viruses (A, B, C) are distinguished by their internal group-specific ribonucleoprotein. Only influenza A and B are medically significant since epidemics or pandemics have not occurred with influenza C. Influenza A has the potential to produce pandemics because it infects other species apart from humans (for example, birds, pigs and horses). In contrast is influenza B, which infects only humans, and so its antigenic structure does not become sufficiently different to cause pandemics. Influenza A viral envelope has two important antigenic glycoproteins—haemagglutinin (HA—composed of 15 different types) and neuraminidase (NA—9 different types) (Figure 8.6). Although the envelope antigens are capable of producing many different combinations (as seen in water birds), a smaller number are found in humans (Table 8.12). To date, only a limited number have been implicated in human-to-human spread (H1N1, H2N2, H3N2, H1N2, H5N1, H9N2 and H7N7).

As the influenza A virus passes through its hosts, the most important of which in terms of global spread are the water birds, it undergoes genetic changes that are of two types: (1) **Genetic (antigenic) drift**—new viral strains frequently result from the accumulation of point mutations in the surface glycoproteins (HA and NA). These mutations are occurring continuously over a period of time. The new strains produced are antigenic variants that nevertheless remain related to viruses from preceding epidemics. Thus, they can avoid immune surveillance in those who have developed immunity, and so outbreaks occur. Because some immunity is still present, only epidemics result. (2) **Genetic (antigenic) shift**—this results from an abrupt major change in antigenicity of the HA protein or the HA and NA protein combination. Either this is the result of a novel HA alone, or in combination with a novel NA. This virus is antigenically distinct from previous ones and has not arisen from them by mutation. It is likely that the sources of the new viral gene are water birds that have a large reservoir of different HA and NA genes. They then get into humans via other animals such as pigs or directly from chickens such as occurred with the H5N1 virus discussed below. The new viral subtype is the precursor to a pandemic because populations have never been exposed to it before (Figure 8.7).

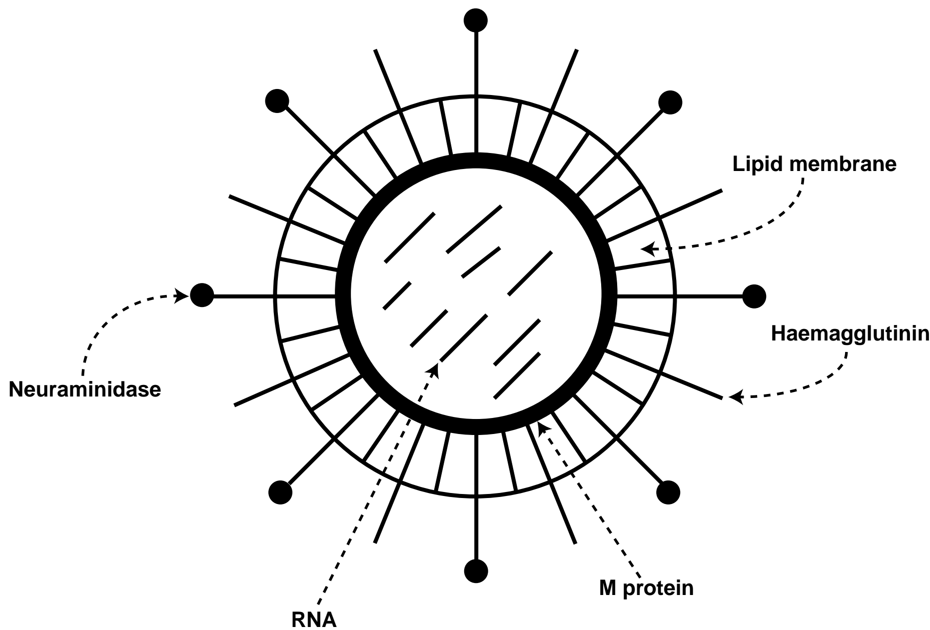

Fig. 8.6 Structure of the influenza virus.
This RNA virus has two key surface glycoproteins: (1) haemagglutinin (HA), which facilitates the entry of virus into host cells through attachment to sialic acid receptors, and (2) neuraminidase (NA), which is involved in the release of progeny virions from infected cells. The HA is the major determinant against which are directed neutralising antibodies and so also the target for influenza vaccines. In contrast, the NA is an important target for antiviral agents.

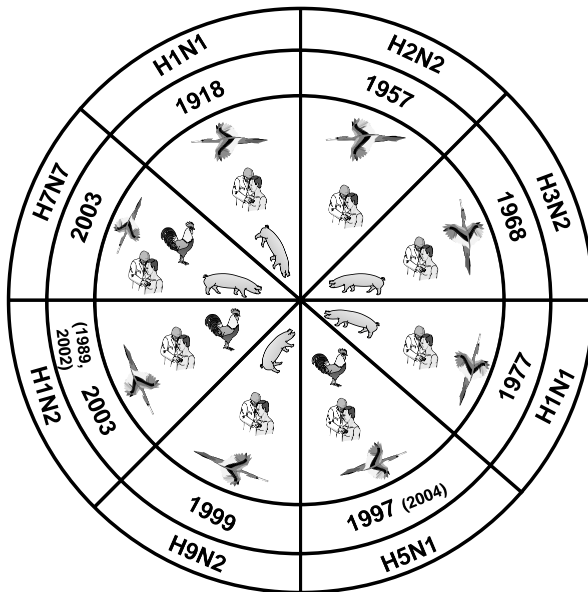

Fig. 8.7 Important animal-to-human and human-to-human influenza outbreaks.
Since the 1918 pandemic, a number of important outbreaks have been recorded (subtypes and dates are given as well as hosts involved). The hosts include water birds, chickens, pigs and humans. A worrying trend is the increasing number of new subtypes in humans, as well as an expanding animal involvement since 1997.

Table 8.12 Species range and types of the influenza A glycoproteins

Animal	Haemagglutinin	Neuraminidase
Water birds[a]	H1–H15	N1–N9
Humans[b]	H1–H3, H5, H7, H9	N1, N2, N7
Pigs	H1, H3	N1, N2
Horses	H3, H7	N7, N8

[a] Wild birds do not usually develop an illness following infection with influenza A. However, domestic birds (chickens, turkeys) can get severe infections, with mortality in some cases (such as the H5N1 subtype) nearing 100%. [b] Subtypes that currently infect humans are H1N1, H1N2 and H3N2.

Avian Influenza (Avian Flu, Bird Flu)

A contemporary worldwide threat to health is the large avian flu epidemic (influenza A—H5N1) presently infecting chickens across many South East Asian countries and mainland China. In 1997, the first cases of human infection from exposure to sick birds or their droppings were reported in Hong Kong. Eighteen patients were admitted to hospital and six died. Fortunately, the culling of more than a million chickens controlled this particular outbreak. Since then, there have been other human cases, with the most recent reported in Vietnam and Thailand.

By late 2004, 44 humans had contracted the avian flu, and 32 of them had died (73% mortality if the infected person was ill enough to be admitted). The "Spanish" (H1N1) flu epidemic in 1918 killed over 20 million people. However, mortality associated with this infection was estimated to be 2.5%, and so considerably less than what is being seen with H5N1 avian flu. It is possible that there have been milder cases of the H5N1 flu that have gone unrecognised, but this worrying comparison as well as the broadening host range for the H5N1 virus (particularly the involvement of the pig) remain important global concerns.

In contrast to the common human influenza virus (H3N2), which is highly contagious but rarely lethal, the avian flu in chickens is a particularly virulent type that can kill rapidly and that causes widespread organ damage. On the other hand, it is not easily transmitted from birds to humans or human to humans. So far only two probable cases of human to human transmission have occurred. However, swapping of genetic material should an individual be co-infected with both might produce a hybrid H5 (avian flu) N2 (human flu) virus with devastating effects. Another way for this mixing to occur (called reassortment) would involve an animal such as a pig being simultaneously infected with both the human influenza virus and the bird flu virus. This mixing might produce a new virulent virus if it had human genes (allowing human-to-human spread) and lethal genes from the bird virus. DNA sequencing of the viral genome from various outbreaks has shown: (1) Viruses from 1997 and 2003 Hong Kong infections have mutated; i.e., their DNA sequences are different. (2) Viruses from the most recent Vietnam and Thailand outbreak show that the virus is resistant to two of the four antiviral agents available. (3) Fortunately, the genes remain of bird origin; i.e., the virus has not acquired genes from human influenza virus, the precursor to a pandemic.

Other outbreaks of bird flu recently reported show subtypes H7N3, H5N2, H7N2, H2N2. These outbreaks have involved poultry in various parts of Canada and the USA. The H7N3 infection resulted in two humans getting a mild flu-like illness following exposure to sick poultry. One person died in New York in late 2003 from an H7N2-type influenza A infection although details of how this occurred are not known. The culling of many birds has controlled the risk of avian flu to humans, but the virus continues to re-emerge in poultry in several Asian countries, with a suggestion that H5N1 might be increasing in pathogenicity and becoming more widespread. Close surveillance by the WHO, the CDC and other bodies continues. Another concern is that clinical features and travel history of individuals who might have SARS (discussed below) and flu virus H5N1 are similar. Hence, vigilance by health professionals and airport monitoring continues, particularly in those with a travel history and respiratory symptoms suggestive of these infections. In this unpredictable environment, the value of rapid DNA-based diagnostics is obvious.

Vaccines

Characterisation of the different viral types and their mode of development depends on the analysis of the HA and NA amino acids, and more recently, the sequencing of the single-stranded RNA genome of this virus. For this purpose, the WHO has a number of collaborating centres that sequence (by RT-PCR) various viral strains, particularly the HA and NA genes, to monitor the changes as they occur. The ability to identify the functional components of the viral ribonucleoprotein complex and then produce these by rDNA means will provide a potential source of antigens for immunisation programs.

Another contribution from molecular medicine has come in developing a vaccine to the potentially serious avian flu virus. Normally, influenza vaccines are prepared in chicken embryos, but since H5N1 is very lethal to chickens, it cannot be grown in this way. An additional step is necessary using recombinant DNA means to take out the H5N1 genes required for the vaccine and place them in a less toxic laboratory virus that will grow in the chicken embryos.

TAXONOMY

Echinococcus

Worldwide, the tapeworm *Echinococcus granulosus* is an important public health problem leading to the development of cysts in many body organs (hydatid cyst disease). An interesting feature of this parasite is the phenotypic heterogeneity observed in the various isolates, including those obtained from different intermediate hosts such as sheep, cattle, goats and camels. The parasite's variability has made it more difficult to understand its life cycle, to distinguish pathogenic from non-pathogenic types and to determine the best approaches to treating animals that are carriers. However, these issues are now less problematic, with DNA studies providing a more objective way to distinguish the different types, including the potential to distinguish the parasites in their intermediate hosts, making it possible to determine which parasitic strains are more likely to be infective for humans.

The DNA-based taxonomic classification of *Echinococcus* has identified nine different genotypes (G1–G9). Some of these genotypes are found in particular animal intermediate hosts; for example, G1 is the sheep form; G2, Tasmanian sheep; G6, camel; G5, cattle; and so on. The horse strain (G4) is not infective to humans. Another strain (G8) is associated with *E. granulosus* infections in Alaska and was not considered to be highly pathogenic until DNA analysis of a severely affected

Box 8.2 Hydatid disease in Alaska (McManus *et al* 2002).

Hydatid disease caused by *Echinococcus granulosus* has two different forms in North America: (1) Domestic (pastoral)—dogs are definitive hosts, and typical intermediate hosts are sheep. Humans get infected through exposure to *E. granulosus* eggs shed by the dogs. (2) Sylvatic—this is found in higher latitudes, and the corresponding hosts are wolves or sled dogs and moose or reindeer. Based on experience of hydatid disease in Alaska, treatment of the sylvatic form is usually conservative rather than surgery since the natural history of hydatid disease in the higher latitudes is fairly benign. Therefore, it was surprising when two patients developed a severe form of hydatid disease in Alaska. One explanation for this different clinical profile was infection from some other geographic region (both had visited the lower United States). However, in one patient, DNA analysis of the *E. granulosus* showed that it was the G8 genotype, i.e., what would normally be found in Alaska. This was confirmed by showing that a number of infected moose carried the identical strain. The case provides further understanding of the G8 variant of *E. granulosus*, which normally follows a relatively benign natural history, but in some unusual cases (for reasons as yet unknown) it can produce severe disease. Until DNA analysis became available, this level of understanding of hydatid disease would not have been possible.

individual showed that this was not the case (Box 8.2).

Anopheles

Anopheles are insects that are medically important because of their association with malaria, filariasis and arbovirus infections. There are nearly 500 recognised species of the *Anopheles* mosquito. However, only a small number are vectors for human diseases. The importance of being able to identify them is illustrated by a mosquito eradication program in Vietnam that failed because the wrong mosquito was targeted. This error was discovered by DNA typing, and it occurred because the female forms of different strains in that particular geographic region were difficult to distinguish, and so a nonvector form of mosquito was mistaken for one involved in the spread of malaria.

Taxonomic classification of these mosquitoes relies on the traditional morphology, to which has been added chromosome analysis, and DNA markers. Recently, the publication of the complete genome sequence for *Anopheles gambiae* has opened up a new source of information that will allow even better characterisation of the *Anopheles* mosquito. There is also the potential for microarray studies of gene expression to understand better the mosquito's metabolic pathways and so expand the options for therapeutic targets.

Fungi

Fungi are eukaryotes that belong to a separate kingdom from plants and animals. They are ubiquitous in the environment and, in addition, have many commercial applications. In humans, infections caused by fungi (mycoses) can be superficial (e.g., skin, hair, nails) or deep (e.g., pneumonia, systemic infection, septicaemia). At particular risk are the immunocompromised for whom mycoses can be life-threatening. Treatment options in fungal diseases are limited. Until recently, there were few medically important fungi. Today, many new infectious opportunistic fungi are emerging.

The large diversity in fungal morphology, their ecological habitats and the wide spectrum of clinical consequences have complicated diagnostic approaches and our understanding of infections caused by these organisms. An important objective in studying fungi is to deduce evolutionary comparisons and, from them, determine relatedness. Fungal phylogenetics relies on parameters described earlier such as morphology, biochemistry and staining characteristics. In addition, life cycles (e.g., morphological appearances, particularly of the sexual reproductive structures) provide additional sources of information for comparison. However, traditional typing methods are not always helpful or sensitive enough and even life cycles can be uninformative; e.g., the presence of asexual forms of *Coccidioides immitis* has made classification particularly difficult.

An early breakthrough in fungal taxonomy based on DNA typing was the finding that *Pneumocystis carinii*, a very common and serious infection in AIDS as well as in other immunocompromised patients, is a fungus and not a protozoan as considered initially. Culture of human-derived *P. carinii* was always difficult. Its life cycle and metabolic processes were poorly understood. However, based on rRNA and DNA sequence comparisons, this organism is now considered a fungus. Today, there are new opportunities in fungal research as a result of the number of organisms that have had their genomes sequenced. This started with the *S. cerevisiae* yeast genome project that was completed in 1996, and there are many others in progress.

Genomics approaches are now very much part of basic research into fungi, and so microarrays (both genomic and protein) are being applied to study important pathogens. By cataloguing the gene profile of these organisms, it is expected that virulence pathways can be identified and then targeted by specific therapies. In an

Table 8.13 Water supply and nosocomial infections (Merlani and Francioli 2003)

Organism	Traditional sources of infection	New source of infection from DNA analysis
Pseudomonas aeruginosa	In intensive care units, *P. aeruginosa* infections can originate from the endogenous gut flora of the patients.	Genotyping of the organisms showed in some studies that the taps in the patients' rooms were the source of infection in more than a third of cases.
Mycobacteria spp.	Non-TB forms of mycobacteria can cause a wide range of infections including abscesses from the use of catheters or endoscopes, the surgical sites or sites related to dialysis.	Non-sterile ice and the inappropriate uses of tap water (rinsing of instruments) have been shown by DNA typing to be another source of these infections.
Fungi	Aspergillus is a life-threatening infection in immunosuppressed patients and is thought to be caused by fungal particles in the air.	In a three-year prospective study, aspergillus was detected in a hospital's water system. Higher titres were found in bathrooms. Genotyping one isolate of *Aspergillus fumigatus* from an infected patient showed that this fungus was the same as isolates recovered from the shower wall in the patient's room.

epidemiologic sense, an understanding of virulence pathways facilitates the investigation of disease outbreaks, particularly nosocomial ones, since it allows a mix of organisms that are likely to be present to be accurately typed and, from this, determine which strains or subtypes are responsible for the infection.

NOSOCOMIAL INFECTIONS

Nosocomial (hospital acquired) infections include bloodstream infections, pneumonia, ventilator-associated pneumonia, intra-abdominal sepsis, wound infections and infections in immunocompromised patients. Pathogens causing these infections are often resistant to many antimicrobial agents. Suboptimal treatment within the hospital environment only exacerbates the problem of drug resistance. In one study of intensive care patients, the effect of bloodstream (bacteraemia and septicaemia) nosocomial infections increased the stay in intensive care units as well as the associated financial costs.

It is important in nosocomial infections to have methods to type pathogens to enable their source(s) and mode of spread to be identified. A composite of DNA polymorphisms will provide a unique DNA pattern for an individual person. DNA fingerprinting, as it is popularly known, is now well established in forensic practice (see Chapter 9). The DNA/RNA profile of infectious agents is similarly being exploited for diagnostic or epidemiological purposes. DNA markers can be selected from a conserved region of the microbial genome if less discrimination is required. On the other hand, choosing a DNA probe from a highly variable (i.e., polymorphic) region of the genome will allow discrimination between closely related organisms. The ultimate in DNA fingerprinting is now available in infectious diseases, and this is the use of DNA sequencing including the option to sequence the pathogen's entire genome.

Legionella

Legionella spp. are ubiquitous bacteria present in domestic and industrial water systems, tanks and other sources of pooled or collected water. About 45 different species of the genus *Legionella* are described. It is now considered to be a relatively common pathogen involving both community acquired as well as nosocomial legionellosis. Infection with this organism is easily overlooked because traditional diagnostic tests are not sensitive enough, particularly with species other than the common *L. pneumophila*. Although air conditioners and cooling towers are recognised sources of this organism, more recently the finding of *Legionella* spp. in drinking water provides another explanation for sporadic or larger outbreaks.

Legionella spp. are found in the water supply of many hospitals. In one study, 17 of 20 hospitals tested were positive, and genotyping showed that each hospital had its own different *Legionella* spp. It is considered that water-borne *Legionella* causes infection when contaminated aerosols are inhaled or there is aspiration of contaminated water. The link between hospital water supplies and various other types of nosocomial infections is slowly growing as DNA typing provides the evidence for similarity between the infecting organisms and those found in the water supply (Table 8.13).

A proportion of cases with Legionnaire's disease is considered to be nosocomial in origin. Both sporadic cases and outbreaks of this respiratory disorder have been reported. The organism (e.g., *L. pneumophila*) is difficult to grow, and so it is frequently necessary to obtain bronchial aspirates or lung biopsies for direct detection. Serological testing is the mainstay of diagnosis although a number of DNA-derived tests are now being developed. These tests can utilise DNA hybridisation with probes that are often rRNA-specific. Another useful rDNA approach to fingerprinting bacterial genomes such as *Legionella* spp. is pulsed field gel electrophoresis (PFGE).

The technique allows large segments of DNA to be separated by using restriction enzymes such as *Not*I and *Sfi*I that digest DNA very infrequently. The upper limit for resolution in conventional DNA gel electrophoresis is approximately 30 kb, whereas for pulsed field gel electrophoresis it is approaching 10 Mb (10×10^3 kb). Pulsed field gel electrophoresis has been particularly attractive in microbiological work because the genomes of infectious agents are relatively small. For example, total DNA from *Legionella* spp. can be cleaved into a limited number of fragments (5–10 with *Not*I, depending on the strain), and these fragments or fingerprints can be directly visualised from an ethidium bromide stained gel, thereby avoiding the requirement for a DNA probe and a hybridisation step. However, the technique is very demanding. Not surprisingly, PCR-based approaches are increasingly becoming the preferred method for DNA fingerprinting. Typing of *Legionella* spp. for epidemiological purposes follows similar strategies.

Methicillin Resistant Staphylococcus Aureus *(MRSA)*

The first isolate of methicillin resistant *S. aureus* was reported in 1961, one year after methicillin became available. This was followed by the emergence of vancomycin-resistant MRSA in 1996. MRSA developed when *S. aureus* acquired a large segment of DNA (called SCC*mec*) that integrated into the *S. aureus* chromosome. This segment of DNA looks like an antibiotic "resistance cassette" because it contains genes for β lactam resistance and genes for resistance against non–β-lactam antibiotics. Vancomycin resistance is thought to have occurred with the emergence of a strain of *S. aureus* that had a thickened cell wall, thereby preventing the vancomycin from getting into the pathogen.

Antibiotic-resistant *S. aureus* is an important and frequent cause of nosocomial infections in hospitals. When this occurs, infection control protocols need to identify the source(s) of the methicillin-resistant *S. aureus* and then determine whether there has been a breakdown in infection control practices so that more effective preventative measures can be implemented in future. For the above to occur, the different bacterial subtypes need to be distinguished. Phage typing has been the traditional tool in epidemiological studies involving *S. aureus*. However, this method has poor reproducibility, it is not able to type all isolates and it is impractical for most laboratories since it requires a large number of phage stocks to be maintained. Phage typing is now being replaced by DNA-based tests. Like the example of *Legionella*, MRSA typing can involve a wide range of techniques from PFGE to PCR. Although these techniques are often more discriminatory than the traditional approaches, they now need to be standardised to allow better inter-laboratory comparisons to be made.

EMERGING INFECTIONS

Emerging infections describe infections that are newly identified or whose incidence in humans has significantly increased in the past 20 years. Many factors contribute to the emerging infections, including (1) changes in human behaviour, (2) globalisation (i.e., increased travel, tourism), (3) technologic advances, economic development and changes in the environment and (4) lapses in public health measures including those resulting from poverty and war. Very few of the emerging infections represent novel pathogens. Most are the result of a change in the epidemiology or virulence of a pathogen, or secondary to microbial adaptation. Important sources of emerging infections are animals.

ZOONOSES

In the past, the emergence of infectious agents reflected changed patterns of human movements that disrupted traditional geographical boundaries. For example, yellow fever is thought to have emerged in the New World as a result of the African slave trade, which brought the mosquito *Aedes aegypti* in ships' water containers. More recently, *Aedes albopictus*, a potential vector for dengue virus, has become established in the USA following its conveyance from South East Asia in old car tyres. With this, the threat of dengue in the North American continent has become real. Most emergent viruses are zoonotic; i.e., they are acquired from animals, which are reservoirs for infection. Thus, completely new strains are less likely than the appearance of a virus following a change in animal reservoirs. This is particularly relevant to the modern world where the consequences of easy migration, deforestation, agricultural practices, dam building and urbanisation are making, and will continue to have, a major impact on the ecology of animals. At the same time, humans have increasingly populated rural areas and are pursuing more outdoor recreational activities. There is also a growing trend for keeping exotic animals as household pets. Changes in global climate may also contribute directly, through their effects on vegetation, insect and rodent populations.

Table 8.14 lists a number of zoonoses that have become established as new infectious diseases or are emerging as problems for the future. Some of them are newly acquired in the West, whereas others remain endemic to specific countries. In many cases, molecular

Table 8.14 Examples of zoonoses and new human infections

Pathogen	Clinical problems	Epidemiology	Emergence	Role of DNA technology
West Nile Virus RNA Flavivirus related to Yellow fever; Japanese encephalitis	Febrile illness complicated by meningoencephalitis, weakness and paralysis.	Transmitted by mosquitoes; blood or organ donation; pregnancy, lactation; infected needles or laboratory specimens. The virus is maintained by a bird-mosquito-bird cycle.	Isolated in 1937 from Uganda and found in many parts of the world. Appeared in the USA in 1999 and has rapidly spread across North America.	NAT is used to screen blood donors and is potentially useful for immunosuppressed individuals who cannot mount an antibody response for serologic testing.
Monkeypox virus DNA virus related to smallpox	Self-limited febrile illness with vesiculo-pustular eruptions. Can be confused with more serious illnesses.	Primary animal reservoir is the rat.	Recognised in 1958 and remained localised to Africa. In 2003, outbreak in Midwest USA shown to be monkeypox. Traced back to rats imported from Africa to which native prairie dogs were exposed and became infected and then infected humans. Appears to be contained.	DNA characterisation helped in identifying this virus as monkeypox.
Ebola RNA filovirus	Haemorrhagic fever in humans (mortality 20–100%)	Example of increased human-to-animal contact in tropical forest. Animal host remains unknown (bats are suspected).	First isolated in 1976 from Sudan and Zaire. Since then, sporadic outbreaks have occurred. Remains isolated to Africa.	PCR-based assays for rapid and sensitive diagnostic tests described.
Lassa fever RNA virus	Haemorrhagic fever with 20% having severe multisystem disease.	Rats infect humans (by contact or if eaten). Virus excreted in human urine/semen for months after infection.	Endemic in West Africa since 1950s.	Nil
Hantavirus RNA virus	Haemorrhagic fever with renal syndrome and pulmonary syndrome (severe disorders).	Infection occurs through exposure to rodent excreta.	Isolated in 1979 in Korea. Now established within the Eurasian continent. Outbreaks in the USA in 1993 and 1998 thought to be due to climatic changes, increasing vegetation and so an increase in the rodent population. Now established throughout North Central and South America.	The types of Hantaviruses in different locations show a close relationship to their rat host. But now shown by DNA analysis that hantaviruses can jump from one rat species to another. This will make it more difficult to know from studying the rat populations if a particular type is likely to be virulent.
Lyme disease Bacterial spirochaete *Borrelia burgdorferi*	Early non-specific malaise can be complicated by arthritis, neurologic and cardiac problems.	Tick (*Ixodes* spp.) transmitted disease. Mice, rodents and birds are the intermediate hosts.	First recognised in the USA in 1957; since then reported in many countries.	DNA characterisation useful for epidemiologic purposes and to explain different clinical features in various countries.
HIV RNA lentivirus	Serious acquired immunodeficiency disorder.	Cross-species transmission from non-human primates followed by human-to-human spread.	Evidence for the link between non-human primate and human disease includes (1) similar viral genomes, (2) prevalence in the natural host, (3) geographic co-location.	DNA technology has been helpful in all phases of this particular disease from diagnosis to prognosis (in terms of viral load determination and detection of viral resistance).

Table 8.14 *Continued*

Pathogen	Clinical problems	Epidemiology	Emergence	Role of DNA technology
BSE, CJD, vCJD Bovine spongiform encephalitis, Creutzfeldt-Jakob disease and variant CJD	Fatal spongiform encephalitis with a long incubation peiod.	BSE detected in mid-1980s in cattle, resulting from transmission of prions in meat and bone meat products used as animal feed in the UK.	vCJD, the human equivalent of BSE, identified from 1996 due to the ingestion of meat from infected cattle. Theoretical risk that it can be transmitted through blood, although not proven.	Infectious agent does not have nucleic acid and so not detectable by conventional means. DNA analysis has shown mutations in the *PRNP* gene (see also Figure 8.8 and Chapter 4).
PERV Pig endogenous retrovirus	Xenotransplants (especially from pigs) considered as a source of organs or cells because there are insufficient human donors.	Concern that endogenous pig retroviruses (PERV) might jump the species barrier particularly if immunosuppression is needed for the xenotransplant.	Theoretical risk based on *in vitro* evidence that PERV can infect human cell lines. Nevertheless, a concern holding back xenotransplantation.	DNA testing can detect PERV but does not necessarily indicate infectivity.

medicine offers little to current management including diagnosis, although much knowledge about these infections has come from analysis of the organisms' DNA or RNA. However, the potential for spread of any disease is high because of international travel or the mass dislocation of large populations following civil unrest. There is also an increasing awareness that a number of pathogens could be used for bioterrorism. Some of the zoonoses associated with a viral haemorrhagic clinical picture can be confused with other clinical infections including malaria, leptospirosis and *Neisseria meningitidis,* and in these potentially fatal conditions, a rapid screening test is essential. In terms of bioterrorism and the differential diagnosis of haemorrhagic fevers, PCR (NAT) based assays are presently the only options with the potential to allow rapid and sensitive diagnostic tests to be developed.

SARS

SARS (severe acute respiratory syndrome) attracted a lot of publicity and provoked fear in 2003. It has been described by some as the first pandemic of the twenty-first century since it involved over 8000 patients and caused more than 800 deaths in 30 countries on five continents (Peiris *et al* 2003). The economic impact of this infection in Hong Kong was estimated to be nearly US$6 billion. SARS also represents a contemporary paradigm that illustrates the value of molecular-based DNA approaches in dealing with a serious emerging infection. The journal *Science* acknowledged the impressive work undertaken to control SARS, citing it as the outstanding achievement of the year: "SARS: a pandemic prevented" (see Table 1.1). A chronology of events in the SARS story is given in Table 8.15.

Traditional approaches such as viral culture, electron microscopy and serology played a key role in characterising the SARS virus now shown to be a coronavirus (CoV). Nevertheless, the molecular approach enabled the following key information to be obtained in a very short time frame:

- Molecular typing of this virus from an outbreak in Taiwan confirmed that the virus was identical in its DNA sequence to a virus isolated from a Hong Kong outbreak; i.e., human-to-human spread had occurred through an individual who had been in Hong Kong. Similar comparisons were possible, linking various outbreaks through molecular epidemiology.
- Rapid whole genome sequencing of the viral RNA provided a firm base for the development of PCR-based diagnostic assays, a critical requirement for acute infections such as SARS-CoV.
- In searching for animal reservoirs, RT-PCR–based techniques were used. They enabled the SARS-CoV to be detected, as well as identified genetic differences between the human and animal virus.
- Future understanding of the virus, how it evolved, what strains were pathogenic and its mode of spread to populations could be addressed through DNA approaches. For this, the US National Institute of Allergy and Infectious Diseases released a genome chip containing the full 29 700 base pairs of the SARS viral genome.

The SARS outbreak ended just as quickly as it started. Only the occasional cases were reported in early 2004, and none was reported after the end of April that year. However, there remain many unanswered questions, including the inconsistent human-to-human transmission with documented cases of one infected passenger transmitting to a number of others on a particular flight, while on another flight no transmission occurred despite a number of passengers being ill with this virus. These inconsistencies were explained by proposing that some

Table 8.15 Chronology of events in the SARS story

Date	Event
November 2002	Atypical pneumonia cases in Guangdong Province, China.
January 2003	Outbreaks of pneumonia in same province.
February 11, 2003	WHO notified about outbreaks of pneumonia in Guangdong Province. At this stage concern that this represents influenza A (H5N1) cases identified in Hong Kong.
February 21, 2003	Resident from Guangdong Province checks into Hong Kong hotel. He becomes ill and is hospitalised. Subsequently shown that he has infected at least 17 other visitors/guests at the hotel (they will later travel to Vietnam, Singapore and Toronto to spread SARS locally).
February–March 2003	Contacts at Hong Kong hotel become ill, and some infect health care workers. Hotel contact dies in Toronto.
March 12–14, 2003	WHO issues global alert; clusters of atypical pneumonia are reported in Singapore and Toronto (later, it will become evident that these clusters are linked to the Hong Kong hotel).
March 15, 2003	The atypical pneumonia is called SARS; more than 150 new cases are reported; WHO issues a travel warning.
Mid-late March 2003	Novel coronavirus isolated from SARS patients.
May 12, 2003	The complete sequence of the coronavirus is announced; virus now called SARS-CoV.
June 2003	Virus related to SARS-CoV isolated from animals.
July 2003	Official end of the SARS outbreak in humans.
August 2003	The identification of SARS-CoV as the cause of an outbreak of mild respiratory infection in a Canadian nursing home is recanted. Error based on laboratory misdiagnosis.
September 2003	Laboratory-acquired SARS-CoV infection (mild) reported in Singapore.
October 2003	Concern expressed that the definitive animal reservoir for SARS-CoV remains unknown and so future outbreaks are possible. Virus detected in civet cats and a raccoon dog in a Chinese market. These are a likely source for the human infections, but the source for these animals remains unknown.
December 19, 2003	Breakthrough of the Year by *Science* magazine: SARS: A pandemic prevented.
January–April 2004	In January, two suspected cases of SARS-CoV confirmed in Guangdong. The last cluster of nine cases (one fatal) reported in China in April. No further cases of SARS described after April 29, 2004.

of those infected were "super-spreaders." Another intriguing observation with SARS is the relatively large numbers of health workers that have been infected; i.e., SARS transmission seems to be more likely within a hospital setting.

Although a number of emerging viruses have been described recently, SARS-CoV is particularly worrying because it is one of the few that has shown human-to-human transmission, although fortunately this seems to be relatively inefficient. For the future, a key issue will be early and prompt recognition of new cases to enable traditional public health measures (the reason for effective containment on this occasion, although the seasonal changes may also have contributed) to be implemented even faster. The WHO has also flagged the importance of laboratory containment when dealing with this virus. This became an issue when two of the nine persons infected in China in 2004 worked in a reference laboratory conducting research into SARS-CoV. A similar scenario was reported earlier from Singapore. The latter case was clearly documented on DNA sequencing of the virus to be due to a contaminated laboratory culture that the scientist had been working with three days before showing signs of the infection.

An issue that is still outstanding with SARS-CoV is how this virus is normally spread. Communicable respiratory infections traditionally infect others because of droplet spread or contact with contaminated surfaces. Some preliminary data are now being reported that SARS-CoV involves airborne transmission. If proven, this will be an interesting public health challenge requiring a better understanding of this mode of transmission, including its relationship to modern housing, travel and working environments. Detecting viral particles involved in airborne spread will not be easy, and for this PCR will become an indispensable tool for the public health professional.

BIOTERROR

After the 2001 anthrax bioterror cases in the USA, the world has been placed on alert. A new form of modern terrorism had been launched. In Chapter 9 reference is made to the US government's move to develop "microbial forensics" as a way in which to deal with bioterrorism or biocrimes involving the use of human or animal pathogens. DNA technology will be a particularly valuable asset in microbial forensics, allowing the rapid identification of various pathogens, and as part of the detective work, to find the source of the infection. As discussed further in Chapters 1 and 9, it is fortuitous that a component of the Human Genome Project was the sequencing of genomes from a number of pathogens. This information will be needed in combating bioterrorism.

Biological warfare or bioterrorism is possible through many ways, including the contamination of food or water

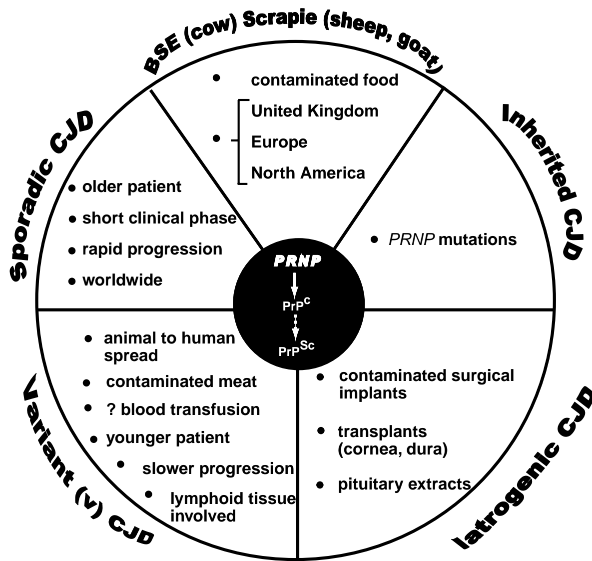

Fig. 8.8 BSE, CJD and vCJD.
BSE (mad cow disease) is caused by a prion, i.e., abnormal form of a protein (PrP) that can spread from one organism to another and interfere with the shape of the normal protein. The gene producing the PrP protein is called *PRNP*, and the two forms are PrP^{c} (normal protein; the c = cellular) and PrP^{Sc} (abnormal protein; the Sc = scrapie). In the UK, BSE resulted from the feeding of contaminated food to cattle. A similar disease is found in sheep and goats (called scrapie). In humans, the same disease is called CJD (Creutzfeldt-Jakob disease). There are a number of forms of CJD. The sporadic type is rare and occurs in the elderly. The inherited form is also rare, and it can be shown to have mutations in the *PRNP* gene. Iatrogenic CJD occurs from contaminated instruments or human products. Because the prion is not a traditional pathogen, sterilisation methods are not effective. Variant CJD (vCJD) is the result of direct animal-to-human spread (through contaminated beef products). vCJD has not been transmitted by blood, but because of the long incubation period possible (perhaps 30 or more years), this cannot be excluded, and some disturbing experimental data suggest that this is possible.

Box 8.3 Bioterrorism using anthrax (Griffith *et al* 2003).

Anthrax is caused by *B. anthracis*, a gram positive spore-forming organism. It is usually acquired by humans through exposure to infected animal products or contaminated dust. The major forms of anthrax are cutaneous (95% of cases, with a mortality of about 20%) and pulmonary (100% mortality if not treated before symptoms develop). In October and November following the September 11, 2001, World Trade Centre disaster, 7 cutaneous and 11 inhalational cases of anthrax occurred in Florida, New York City, New Jersey, Washington DC and Connecticut. They were examples of bioterrorism since all bacteria came from the one source based on DNA typing, which showed an identical genotype. The likely route of infection was intentionally contaminated mail. Apart from two cases, all others were mail or government workers, or employees at news media outlets. The two exceptions were a 61-year-old woman in New York City and a 94-year-old woman in Connecticut. Both died after developing inhalational anthrax. It was possible to detect a likely source of infection in the case of the Connecticut victim. The postal processing and distribution centre (PDCs) for the letter-related cases had been identified through the finding of contaminating bacteria. However, for this victim, no samples from her home or personal items tested positive for *B. anthracis*. It was known that she had received mail (including junk or bulk mail) from one of these PDCs. It was also subsequently shown that her local PDC (Southern Connecticut) had a heavily contaminated sorting machine. From this, it was assumed that *B. anthracis* identical to those involved in the other outbreaks had infected her through a letter directly contaminated in a PDC implicated in the earlier infections, or secondarily contaminated in the Southern Connecticut PDC.

supplies, or via infected animals including insects. A more serious form would involve the use of aerosols containing the pathogens because now there is the risk for infecting a very large number of people. The length of the incubation period is also a consideration since it would, to some degree, influence the number infected before containment or treatment was initiated. Biosensors are being developed to assist early detection. In some infections, DNA-based chip technology is being tried as a way in which microbes can be detected directly through their DNA sequence. This will ensure rapid detection and typing of pathogens.

Two pathogens of particular relevance to bioterror are anthrax and smallpox. DNA-based genotyping has already proven its value in the case of the 2001 US anthrax cases (Box 8.3). Promising reports now suggest that a pathogen such as anthrax could be detected within an hour by real-time PCR. Smallpox is another serious infection since routine vaccination was stopped in the USA in the early 1970s, leaving many unvaccinated and so vulnerable targets in this and other countries. In Asia, case fatality rates of around 30% were observed during epidemics, and there are no known treatments for smallpox. As part of its biologic warfare program, the former Soviet Union produced smallpox, anthrax and other pathogens, and it remains a concern that some could fall into the hands of potential terrorists.

FUTURE

Over the past century, there has been a steady decline in infectious diseases in affluent countries, and these diseases have become relatively minor problems when compared to the increasing role played by chronic and neoplastic diseases. During the 1980s this situation started to change with the onset of AIDS and its related infections such as tuberculosis. Other emerging infections have appeared and are important concerns for the future.

The hepatitis C virus is an example of a pathogen that is starting to impact on the health and well-being of many communities. Although it does not have the same emotive force as HIV, it is an important modern scourge that will need to have its spread controlled more effectively, and those infected will need access to antiviral agents (Box 8.4).

A key to controlling the spread of modern pathogens will be effective vaccines that are developed on the basis of the organism's genome sequence (some call this reverse vaccinology). This is illustrated by the group B meningococcus for which a vaccine could not be developed by traditional means. However, soon after the genome was sequenced, it was possible to identify many potential vaccine candidates that are now being tested in clinical trials. The genomic era has opened up new opportunities for the control of infectious diseases.

The SARS example also illustrates the value of genomics in public health medicine. In particular, the very rapid sequencing of the genome of what appeared to be a coronavirus-like pathogen enabled a definitive diagnosis to be made and potential vaccine targets to be identified, some of which are already in the animal phase of testing.

Developments in technology are addressing many of the potential problems related to DNA testing in microbiology. A challenge for the future will be a better understanding of what the finding of a DNA (or RNA) sequence means in terms of infectivity and pathogenesis. The traditional approach has many years of experience to help in interpreting a result such as a positive culture. The same experience is not available with DNA testing, but it will be necessary to do the hard work and determine the significance of various DNA tests. An example would be persistence of mycobacterial growth in culture despite treatment. This would suggest a compliance issue or the development of drug resistance. The finding of a persistently positive DNA test in this circumstance would be more difficult to interpret. The validation of positive DNA test results will be necessary for a wide range of infections; for example, the finding of CMV DNA by PCR in a patient's serum could mean active disease or latent infection. How these issues are resolved will be important for the integration of molecular diagnostics into routine microbiological laboratory practice.

No doubt the potential for genomics will allow novel ways to detect infection with pathogens. One early model described involves the use of microarrays to detect gene profiles in the patient rather than attempts at detecting pathogen-specific nucleic acids. Using this approach, it was claimed that peripheral blood mononuclear cells exposed to *M. tuberculosis*, a protozoan (*Leishmania donovani*) and a worm (*Brugia malay*) demonstrated discrete expression profiles particular to the infectious agent.

Box 8.4 Hepatitis C virus (HCV).

Thirteen years after the term "non-A, non-B" hepatitis was first used, the HCV virus was found. A milestone in molecular virology came when HCV was cloned in 1988, years before it was visualised, cultured or even characterised. Based on its amino acid sequence and genomic organisation, HCV was classed in a separate genus in the viral family *Flaviviridae*. HCV is an RNA virus and has six genotypes (1–6). The main source of infection today is intravenous drug use. Transfusion-related HCV is rarely seen because of the effective screening methods in place. Unlike HBV, mother-to-infant and sexual transmission is rare. Nearly 170 million people worldwide are infected with HCV, which is now the most common reason for liver transplantation in the USA. Unlike HBV, which infrequently leads to chronic hepatitis, about 70% of those infected with HCV develop this complication. Like HBV, the hepatic damage caused by HCV is thought to be secondary to an immune response rather than a direct viral effect. In addition, age, male sex, alcohol consumption, coexistent HIV infection, low $CD4^+$ count and metabolic disorders such as diabetes predispose to the development of liver cirrhosis and subsequently hepatocellular cancer. Like HIV, excellent serologic or DNA-based assays are available for accurate diagnosis. Quantitative RNA assays and genotyping enable predictions to be made of how an individual will respond to antiviral therapy. Treatments using interferon alpha and ribavirin are effective, particularly with HCV genotypes 2 and 3 (88% response rates compared to 48% for the other four genotypes). However, because of inadequate detection (or the money to pay for the above drugs), there is considerable concern that what is being seen at present is only the tip of the iceberg since the virus has a long incubation period, and many who are carriers remain asymptomatic until complications develop.

FURTHER READING

Diagnostics

Domig KJ, Mayer HK, Kneifel W. Methods used for the isolation, enumeration, characterisation and identification of *Enterococcus* spp. 2. Pheno- and genotypic criteria. International Journal of Food Microbiology 2003; 88: 165–188 (*provides overview of the different phenotypic and genotypic assays used in various microbiological applications*).

Allain J-P. Genomic screening for blood-borne viruses in transfusion settings. Clinical Laboratory Haematology 2000; 22: 1–10 (*comprehensive overview of NAT technology as well as effects it has had on HIV, HCV and HBV*).

Clavel F, Hance AJ. HIV drug resistance. New England Journal of Medicine 2004; 350:1023–1035 (*comprehensive review of this topic*).

Forster SM. Diagnosing HIV infection. Clinical Medicine 2003; 3:203–205 (*succinct summary of diagnostic tests for HIV AIDS*).

Fraser CM. A genomics based approach to biodefence preparedness. Nature Reviews Genetics 2004; 5:23–33 (*lists all the pathogens that have been sequenced*).

Gilbert GL. Molecular diagnostics in infectious diseases and public health microbiology: cottage industry to postgenomics. Trends in Molecular Medicine 2002; 8:280–287 (*in-depth analysis of infectious disease diagnostics*).

Goodnough LT. Risks of blood transfusion. Critical Care Medicine 2003; 31:S678–S686 (*excellent overview of all risks associated with blood transfusion*).

Louie M, Louie L, Simor AE. The role of DNA amplification technology in the diagnosis of infectious diseases. Canadian Medical Association Journal 2000; 163:301–309 (*useful overview of what DNA diagnostics can achieve*).

Pealer LN *et al*. Transmission of West Nile virus through blood transfusion in the United States in 2002. New England Journal of Medicine 2003; 349:1236–1245 (*describes the West Nile virus and transmission through blood*).

Strohl WA, Rouse H, Fisher BD (Harvey RA, Champe PA series editors). Lippincott's Illustrated Reviews: Microbiology. 2001. Lippincott Williams & Wilkins, Philadelphia (*well-presented overview of many topics in infectious disease*).

http://www.aidsmap.com (*UK-based charity web site providing a vast array of data on HIV-AIDS*).

http://www.who.int/whr (*World Health Organization 2004 Report on AIDS*).

Pathogenesis

Buer J, Balling R. Mice, microbes and models of infection. Nature Reviews Genetics 2003; 4:195–205 (*summarises the utility of mouse models in infectious diseases*).

Carrington M, O'Brien SJ. The influence of HLA genotype on AIDS. Annual Review of Medicine 2003; 54:535–551 (*summary of various HLA associations with infections*).

Chinen J, Shearer WT. Molecular virology and immunology of HIV infection. Journal of Allergy and Clinical Immunology 2002; 110:189–198 (*provides relevant overview of HIV and AIDS*).

de Viedma DG. Rapid detection of resistance to *Mycobacterium tuberculosis*: a review discussing molecular approaches Clinical Microbiology and Infection 2003; 9:349–359. (*useful summary of resistance genes with a comprehensive overview of the molecular diagnostics technologies*).

Ganem D, Prince AM. Hepatitis B virus infection—natural history and clinical consequences. New England Journal of Medicine 2004; 350:1118–1129 (*useful review of the hepatitis B virus*).

Lage H. ABC transporters: implications on drug resistance from microorganisms to human cancers. International Journal of Antimicrobial Agents 2003; 22:188–199 (*reviews the multidrug transporters*).

Le Bras J, Durand R. The mechanisms of resistance to antimalarial drugs in *Plasmodium falciparum*. Fundamental & Clinical Pharmacology 2003; 17:147–153 (*comprehensive report on molecular basis for drug resistance*).

Lin J-C *et al*. Quantification of plasma Epstein Barr virus DNA in patients with advanced nasopharyngeal carcinoma. New England Journal of Medicine 2004; 350:2461–2470 (*shows how DNA quantification can help to plan treatment for this tumour*).

Low DE. The era of antimicrobial resistance—implications for the clinical laboratory. Clinical Microbiology and Infection 2002; 8 (suppl 3):9–20 (*describes antibiotic resistance in* Streptococcus pneumoniae).

Mansky LM. HIV mutagenesis and the evolution of antiretroviral drug resistance. Drug Resistance Updates 2002; 5:219–223 (*summarises how drug resistance evolves in HIV*).

Miller LH, Baruch DI, Marsh K, Doumbo OK. The pathogenic basis of malaria. Nature 2002; 415:673–679 (*useful review*).

Moss SF, Sood S. *Helicobacter pylori*. Current Opinion in Infectious Diseases 2003; 16:445–451 (*well-presented overview of this pathogen*).

Munoz N *et al*. Epidemiologic classification of human papillomavirus types associated with cervical cancer. New England Journal of Medicine 2003; 348:518–527 (*lists the various HPV types and their risk for cervical cancer*).

Nizet V. Streptococcal β hemolysins: genetics and role in disease pathogenesis. Trends in Microbiology 2002; 10:575–580 (*comprehensive description of genes involved in the* β *haemolysin effect*).

O'Brien SJ, Nelson GW. Human genes that limit AIDS. Nature Genetics 2004; 36:565–574 (*succinct summary of genetic variants and their possible effects on HIV infection*).

Segal S, Hill AVS. Genetic susceptibility to infectious disease. Trends in Microbiology 2003; 11:445–448 (*succinct overview of the association and linkage study to identify genetic susceptibility factors in infectious diseases*).

Young LS, Murray PG. Epstein Barr virus and oncogenesis: from latent genes to tumours. Oncogene 2003; 22:5108–5121 (*useful summary of EBV and pathogenesis of cancer*).

Waggoner SE. Cervical cancer. Lancet 2003; 361:2217–2225 (*summary of cervical cancer from epidemiology to treatment*).

http://www.who.int/emc/amrpdfs/WHO_Global_Strategy_English.pdf (*The WHO's global strategy for containment of antimicrobial resistance*).

Epidemiology

Hien TT, de Jong M, Farrar J. Avian influenza—a challenge to global health care structures. New England Journal of Medicine 2004; 351:2363–2365 (*brief summary; for regular updates see the CDC web site: http://www.cdc.gov/flu/avian/index.htm*).

Hiramatsu K, Cui L, Kuroda M, Ito T. The emergence and evolution of methicillin resistant *Staphylococcus aureus*. Trends in Microbiology 2001; 9:486–493 (*comprehensive description of the genetic factors for MRSA*).

Krzywinski J, Besansky NJ. Molecular systematics of Anopheles: from subgenera to subpopulations. Annual Review of Entomology 2003; 48:111–139 (*comprehensive review of taxonomy for this important mosquito*).

Lorentz MC. Genomic approaches to fungal pathogenicity. Current Opinion in Microbiology 2002; 5:372–378 (*describes genomic approaches to studying fungi*).

Merlani GM, Francioli P. Established and emerging waterborne nosocomial infections. Current Opinion in Infectious Diseases 2003; 16:343–347 (*interesting summary of nosocomial infections associated with drinking water*).

McManus D *et al*. Molecular genetic characterization of an unusually severe case of hydatid disease in Alaska caused by the cervid strain of *Echinococcus granulosus*. American Journal of Tropical Medicine and Hygiene 2002; 67:296–298 (*brief report on how DNA typing can be used to understand disease heterogeneity*).

Nicholson KG, Wood JM, Zambon M. Influenza. Lancet 2003; 362:1733–1745 (*comprehensive overview of influenza*).

Roig J, Sabria M, Pedro-Botet ML. *Legionella* spp.: community acquired and nosocomial infections. Current Opinion in Infectious Diseases 2003; 16:145–151 (*useful summary of* Legionella).

Webby RJ, Webster RG. Are we ready for pandemic influenza? Science 2003; 302:1519–1522 (*specifically looks at relevant issues and pandemics*).

Emerging Infections

Drosten C, Kummerer BM, Schmitz H, Gunther S. Molecular diagnostics of viral hemorrhagic fevers. Antiviral Research 2003; 57:61–87 (*overview of the type of PCR tests that might be used for viral hemorrhagic fevers*).

Griffith KS *et al*. Bioterrorism-related inhalational anthrax in an elderly woman, Connecticut, 2001. Emerging Infectious Diseases 2003; 9:681–688 (*provides some insight into the investigative approaches that would be required in the case of bioterror*).

Irani DN, Johnson RT. Diagnosis and prevention of bovine spongiform encephalopathy and variant Creutzfeldt Jakob disease. Annual Review Medicine 2003; 54:305–319 (*comprehensive review of CJD*).

Lim PL *et al*. Laboratory acquired severe acute respiratory syndrome. New England Journal of Medicine 2004; 350:1740–1745 (*brief account of how a case of SARS resulted from a laboratory source of infection*).

Ludwig B, Kraus FB, Allwinn R, Doerr HW, Preiser W. Viral zoonoses—a threat under control? Intervirology 2003; 46:71–78 (*excellent summary of zoonoses well worth reading*).

Makino S, Cheun H. Application of the real time PCR for the detection of airborne microbial pathogens in reference to the anthrax spores. Journal of Microbiological Methods 2003; 53:141–147 (*illustrates how real-time PCR could be used for rapid DNA detection of pathogens*).

Pealer LN *et al*. Transmission of West Nile virus through blood transfusion in the United States in 2002. New England Journal of Medicine 2003; 349:1236–1245 (*interesting discussion on the implications of WNV and blood transfusion*).

Peiris JSM, Yuen KY, Osterhaus ADME, Stohr K. The severe acute respiratory syndrome. New England Journal of Medicine 2003; 349:2431–3441 (*excellent overview of the SARS story*).

Petersen LR, Marfin AA, Gubler DJ. West Nile Virus. JAMA 2003;290:524–528 (*comprehensive clinical, epidemiologic and historic overview of WNV*).

Reed KD *et al*. The detection of monkeypox in humans in the western hemisphere. New England Journal of Medicine 2004; 350:342–350 (*useful overview of how this infection was quickly identified in the USA*).

Roy CJ, Milton DK. Airborne transmission of communicable infection—the elusive pathway. New England Journal of Medicine 2004; 350:1710–1712 (*brief editorial on SARS and a possible mode for its spread*).

Future

Lin B, Vahey MT, Thach D, Stenger DA, Pancrazio JJ. Biological threat detection via host gene expression profiling. Clinical Chemistry 2003; 49:1045–1049 (*interesting proposal using host rather than pathogen microarray gene profile for rapid detection of infection*).

Poynard T, Yuen M-F, Ratziu V, Lai CL. Viral hepatitis C. Lancet 2003; 362:2095–2100 (*summary of hepatitis C*).

Gold™, Genomes OnLine Database (http://www.genomesonline.org/ (*this Internet site lists all genome projects that have been completed*).