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.
Chapter 1

**Classification and pathogenicity of microbes**

So, naturalists observe, a flea
Hath smaller fleas that on him prey;
And these have smaller fleas to bite 'em,
And so proceed ad infinitum.

Jonathan Swift, *On Poetry*

The microbial causes of human disease include viruses, chlamydiae, rickettsiae, mycoplasmas, bacteria, fungi and protozoa. Basic features of these are included in *Table 1.1*. Arthropods and worms are discussed in later chapters.

Viruses differ greatly from all the other microbes as they consist essentially of only nucleic acid surrounded by a protein coat (capsid) and contain only one instead of two types of nucleic acid. Once inside human cells, the viruses remove the normal nuclear control of the cells to take over cellular metabolism for the synthesis of new virions. Chlamydiae and rickettsiae are also obligate intracellular parasites, have both DNA and RNA, and multiply by binary fission. Mycoplasmas, bacteria and fungi can be cultured in cell-free media unlike the above intracellular microbes.

Bacterial causes of disease are mainly ‘lower’ bacteria which are unicellular. Multiplication is predominantly by asexual binary fission although biological variation is facilitated in some species by ‘sex’, especially with Gram-negative species such as *Escherichia coli*. Only a few ‘higher’ bacteria cause disease in man, such as *Actinomyces israelii* which are filamentous Gram-positive bacilli.

Protozoa pathogenic to man are divided into three main groups:
1. Sarcodina (amoebae), e.g. *Entamoeba histolytica*
2. Sporozoa, e.g. *Plasmodium falciparum*, *Toxoplasma gondii*
3. Mastigophora (flagellates), e.g. *Trichomonas vaginalis*, *Giardia lamblia*, *Leishmania* and *Trypanosoma* species

**CLASSIFICATION OF BACTERIA**

There are three main groups of bacteria:
1. Bacteria that are readily Gram-stained
2. Acid-fast bacilli
3. Spirochaetes
| Type of Microbe | Nucleic Acids | Multiplication | Approx. Size, μm | Seen by Light Microscope | Cell Wall | Cytoplasmic Membrane | Sensitive to 'Antibiotics' | Other Features |
|----------------|--------------|----------------|-----------------|--------------------------|-----------|---------------------|---------------------------|---------------|
| Viruses        | DNA or RNA   | +              | 0.01-0.3        | No                       | No        | No                  | No                        | Host cell may show inclusions |
| Chlamydiae     | DNA + RNA    | +              | 0.3             | No                       | No        | Yes                 | Yes (e.g. tetracyclines)  | Host cell shows characteristic inclusions |
| Rickettsiae    | DNA + RNA    | +              | 0.3             | Sometimes just visible by special stains | Rudimentary cell wall | Yes (e.g. tetracyclines) | ‘Typhus’ transmitted by arthropods |
| Mycoplasmas    | DNA + RNA    | +              | 0.12-0.3        | Sometimes just visible by special stains | No        | Yes                 | Yes (e.g. tetracyclines) | Pleomorphic cells |
| Bacteria       | DNA + RNA    | ± (Depends on particular species) | 0.5-0.8 long | Yes (Muramic acid usually present) | Yes | Yes | Yes | Rigid cell wall |
| Fungi          | DNA + RNA    | +              | Larger than bacteria (>5 long, >0.5 wide) | Yes | Yes | Thicker than bacterial wall + contains sterol | Yes | No sensitive to anti-fungal drugs | Members of plant kingdom but no chlorophyll |
| Protozoa       | DNA + RNA    | ± (Depends on particular species) | Larger than fungi | Yes | Yes | Yes | Not usually | |
**Bacteria that are Readily Gram-stained**

These are classified into Gram-positive (blue-purple) or Gram-negative (pink-red) cocci or bacilli (*Table 1.2*).

Practical details of the Gram-stain are given in the Appendix to Chapter 2, p. 45. After the application of the methyl violet dye, Gram-positive bacteria stain blue and this colour is retained in spite of decolourization with acetone (or alcohol). Gram-negative bacteria initially stain blue after the methyl violet is applied, but the colour is lost after the application of acetone (or alcohol). They then take up the pink counterstain (saffronin, methyl red or carbol fuchsin).

The reason for the difference in colour after Gram-staining is not fully understood, but it is probably related to the large amount of mucopeptide and teichoic acid in the cell walls of Gram-positive bacteria. The fact that Gram-positive bacteria are more acidic than Gram-negative bacteria may account for their greater affinity for a basic dye. Even more important may be the greater permeability of Gram-negative cell walls which allow the methyl violet–iodine dye complex to diffuse out after treatment with acetone more readily than the cell walls of Gram-positive bacteria.

Within each subgroup, there are aerobic or anaerobic examples. The majority of bacterial pathogens can grow either aerobically or anaerobically, i.e. they are ‘facultative anaerobes’ such as *Staphylococcus aureus* or *Escherichia coli*; in *Table 1.2* these have been included as ‘aerobes’. There are a few bacterial species which are strict aerobes, such as *Pseudomonas aeruginosa*, which will not grow at all anaerobically. Some bacterial species are strict anaerobes, such as *Clostridium tetani* or *Bacteroides fragilis*, which will not grow at all aerobically.

Exceptional Gram-stainable bacteria include *Legionella pneumophila* and *Borrelia vincenti*. *Legionella pneumophila* requires prolonged staining with the counterstain to be seen in tissues, although it appears readily as Gram-negative bacilli in smears made from colonies on agar media. *Borrelia vincenti* is the only spirochaetal pathogen that is easily seen by a Gram-stain.

**Acid-fast Bacilli**

Mycobacterial species are not readily seen by a Gram-stain, although they are weakly Gram-positive bacilli. Ziehl–Neelsen or other acid-fast stains are required for staining these organisms which have cell walls containing abundant lipids. Examples include *Mycobacterium tuberculosis* and *Mycobacterium leprae*.

**Spirochaetes**

Spirochaetes are thin-walled spiralled flexible organisms which are motile by means of an axial filament. They are not seen in a Gram-stain (except *B. vincenti*), but may be seen either by dark-ground illumination microscopy, or in a silver stain under the light microscope. Borrelia spirochaetes in the blood may also be seen in a Giemsa stain.

The three groups of spirochaetes include:

1. *Treponema*
   
   Spirochaetes with regular spirals, approximately 1 μm apart from each other, 5–15 μm long and about 0.2 μm wide, e.g. *Treponema pallidum* (cause of syphilis)
Table 1.2. Simple classification of Gram-stainable bacterial pathogens

| Bacteria          | Genus                  | Species examples                |
|-------------------|------------------------|---------------------------------|
| **Gram-positive Bacteria** |                       |                                 |
| Cocci             |                        |                                 |
| Aerobic (Clusters) | Staphylococcus         | S. aureus, S. albus (S. epidermidis) |
|                   |                        |                                 |
| Aerobic (Chains/pairs) | Streptococcus      | S. pneumoniae, S. pyogenes      |
|                   |                        |                                 |
| Anaerobic         |                        |                                 |
| Bacilli            | Bacillus               | B. anthracis                    |
|                   | Corynebacterium        | C. diphtheriae                  |
|                   | Listeria               | L. monocytogenes                |
|                   | Nocardia               | N. asteroides                   |
|                   | Clostridium            | C. tetani, C. welchii (perfringens) |
|                   | Propionibacterium      | P. acnes                        |
|                   | Actinomycetes          | A. israelii                     |
|                   | Neisseria              | N. meningitidis                 |
|                   | Veillonella            | N. gonorrhoeae                  |
| **Gram-negative Bacteria** |                   |                                 |
| Bacilli            | a. Enterobacteria     | e.g. Escherichia - E. coli      |
|                   | Klebsiella             | K. aerogenes                    |
|                   | Proteus                | P. mirabilis                    |
|                   | Serratia               | S. marcescens                   |
|                   | Salmonella             | S. typhi                         |
|                   | Shigella               | Sh. sonnei                      |
|                   | b. Pseudomonas         | Pseudomonas - P. aeruginosa      |
|                   | c. Vibrios             | Vibrio - V. cholerae            |
|                   | Campylobacter          | C. jejuni                       |
|                   | d. Parvobacteria       | Haemophilus - H. influenzae      |
|                   | Brucella               | B. abortus                      |
|                   | Bordetella             | B. pertussis                    |
|                   | Pasteurella, Yersinia   | P. multocida, Y. pestis         |
|                   | e. Legionella          | Legionella - L. pneumophila      |
|                   | f. Spirillum           | Spirillum - S. minus            |
| Anaerobic         | Bacteroides            | B. fragilis                     |
2. *Leptospira*
   Spirochaetes which have tightly coiled spirals, 5–15 μm long and about 0·1 μm wide. Characteristically, there is often a ‘hooked’ end, e.g. *Leptospira icterohaemorrhagiae* (cause of Weil’s disease).

3. *Borrelia*
   Large spirochaetes, 10–30 μm long and about 0·3 μm wide, with irregular spirals 2–4 μm apart from each other, e.g. *Borrelia recurrentis* (a cause of relapsing fever).

**CLASSIFICATION OF VIRUSES**

The classification of viruses depends on several factors including the type of nucleic acid present, the arrangement of the capsids into a cubical (icosahedral), helical or complex symmetry, the number of capsomeres, the size of the virus particle and whether the virion is naked or enveloped (often indicated by ether resistance or sensitivity, respectively, as well as by electron microscopic appearance). The main viruses causing disease in man are classified in Table 1.3.

One way of memorizing which viruses contain DNA is to remember that ‘PHAD’ is for DNA viruses, with *P* for pox and papova, *H* for herpes, *AD* for adenoviruses. Virtually all the remaining pathogenic human viruses are RNA viruses including the self-explanatory picorna viruses (‘PicoRNA’ viruses).

Some DNA viruses may cause tumours in man. These include papilloma virus causing warts, Epstein–Barr virus causing Burkitt’s lymphoma and associated with naso-pharyngeal carcinoma, and herpes simplex virus which is associated with carcinoma of the cervix (see Jawetz et al., 1980). Other viruses, including certain papilloma viruses, have also been implicated in the aetiology of carcinoma of the cervix.

Retroviruses are RNA viruses which may cause tumours directly or indirectly in man and animals. The most important retrovirus is human immunodeficiency virus (HIV) which is the cause of acquired immune deficiency syndrome (AIDS)—see Chapter 21.

**CLASSIFICATION OF FUNGI**

The fungi causing diseases in man belong to the class ‘fungi imperfecti’. There are four main groups of pathogenic fungi: moulds (filamentous fungi), true yeasts, yeast-like fungi and dimorphic fungi.

1. *Filamentous fungi*
   These grow as long filaments called ‘hyphae’ and the branched hyphae intertwine to form a ‘mycelium’. Reproduction is by spores including sexual spores which are used for identification. Culture in vitro of these fungi on Sabouraud’s medium often shows ‘powdery’ colonies due to the presence of abundant spores, e.g. *Trichophyton mentagrophytes*.

2. *True yeasts*
   These are unicellular round or oval fungi. Reproduction is by budding from the parent cell. Cultures in vitro characteristically show ‘creamy’ colonies, e.g. *Cryptococcus neoformans*.

3. *Yeast-like fungi*
   These are like yeasts since they may appear as round or oval cells and grow by budding. They may also form long non-branching filaments known as ‘pseudohyphae’, e.g. *Candida albicans*. 
Table 1.3. Classification of viruses

| Nucleic acid | Capsid arrangement | Naked or enveloped | Size of virus particle, nm | Number of capsomers | Virus family | Virus examples | Diseases |
|--------------|--------------------|--------------------|---------------------------|---------------------|-------------|---------------|----------|
| DNA          | Cubical (icosahedral) | Enveloped          | 100-200                   | 162                 | Herpes viruses | Herpes simplex, I and II Varicella-zoster Cytomegalovirus | Mucocutaneous herpetic lesions Chickenpox and ‘shingles’ Cytomegalovirus inclusion disease Glandular fever and Burkitt’s lymphoma |
| DNA          | Cubical            | Naked              | 70-90                     | 252                 | Adenoviruses | Over 30 serological types of adenoviruses Adenovirus type 8 | Pharyngo-conjunctivitis Lower respiratory infections in infants Epidemic kerato-conjunctivitis (‘shipyards eye’) |
| DNA          | Cubical            | Naked              | 45-55                     | 72                  | Papova-viruses | Papilloma virus SV 40 type viruses | Warts Progressive multifocal leuencephalopathy |
| DNA          | Complex            | Complex coat       | Approx. 200 × 400         | Pox viruses         | Variola      | Monkeypox | Smallpox (now extinct) Monkeypox (rarely affects man) Vaccinia skin lesions after vaccination Contagious pustular dermatitis—orf Molluscum contagiosum | |
| Nucleic acid | Capsid arrangement | Naked or enveloped | Size of virus particle, nm | Number of capsomers | Virus family | Virus examples | Diseases                                      |
|-------------|--------------------|--------------------|--------------------------|---------------------|--------------|---------------|------------------------------------------------|
| RNA         | Cubical            | Enveloped          | 30–90                    | 50–300              | Toga viruses | Alpha and flaviviruses | Arthropod-borne fevers, e.g. equine encephalitis, yellow fever |
| RNA         | Cubical            | Naked              | 20–30                    | 32                  | Picornaviruses | Enteroviruses—polio —echo —Coxsackie A/B | Poliomyelitis Respiratory and CNS infections Respiratory, CNS and heart infections Colds |
| RNA         | Cubical            | Naked              | 60–80                    | 50–130              | Reoviruses    | Rotavirus (wheel-like shape) | Gastro-enteritis |
| RNA         | Helical            | Enveloped          | 80–120                   | 50–130              | Orthomyxoviruses | Influenza A/B viruses | Influenza |
| RNA         | Helical            | Enveloped          | Approx. 70 x 170         | 90–100              | Paramyxoviruses | Respiratory syncytial virus Mumps Measles | Para-influenza Bronchiolitis, 'croup' and colds Mumps Measles Rabies |
| RNA         | Helical            | Enveloped          | 90–100                   | 90–100              | Bunyaviruses  | California arboviruses | Arthropod-borne fevers |
| RNA         | Unknown            | Enveloped          | 50–300                   | 50–300              | Arenaviruses  | Lassa fever virus Lymphocytic choriomeningitis | Lassa fever Aseptic meningitis |
| RNA         | Complex            | Enveloped          | 80–130                   | 50–300              | Corona-       | Coronaviruses | Upper respiratory infections |
4. *Dimorphic fungi*

These grow as yeast forms in the body and at 37 °C on culture media. They also form mycelia in the environment and on culture media at 22 °C. Several examples of this group of fungi grow intracellularly in reticuloendothelial cells in infected patients, e.g. *Histoplasma capsulatum*.

Fungi can also be classified according to whether they cause superficial or deep mycoses in infected patients and some examples are included in Table 1.4. The deep mycoses most frequently occur in immunocompromised patients. Disease might also arise from the ingestion of mycotoxins in food: aflatoxins may be

*Table 1.4 Classification of fungi*

| Fungi causing superficial mycoses |
|-----------------------------------|
| **Fungi** | **Type of fungus** | **Disease examples** | **Geographical distribution** |
|-----------------------------------|---------------------|----------------------|-----------------------------|
| *Dermatophytes* including *Microsporum*, *Trichophyton* and *Epidermophyton* species | Filamentous | Tinea (ringworm) of skin, nails or hair | Worldwide |
| *Aspergillus niger* | Filamentous | Otitis externa | Worldwide |
| *Candida albicans* | Yeast-like | Oral thrush, monilial vaginitis Intertrigo, nappy rash Paronychia, granulomas in chronic mutocutaneous candidiasis | Worldwide |
| *Malassezia furfur* | Yeast-like | Pityriasis versicolor | Worldwide |

| Fungi causing deep mycoses |
|---------------------------|
| **Fungi** | **Type of fungus** | **Disease examples** | **Geographical distribution** |
|---------------------------|---------------------|----------------------|-----------------------------|
| *Aspergillus fumigatus* | Filamentous | Pulmonary or disseminated aspergillosis | Worldwide |
| *Mucor* | Filamentous | *Mucor mycosis* Madura mycosis (‘Madura foot’) | Worldwide Tropics and subtropics Worldwide |
| *Allescheria boydii* | Filamentous | Septicaemia, endocarditis, bronchial and renal infections | Worldwide |
| *Madurella* species | Yeast-like | | |
| *Candida albicans* | Yeast-like | | |
| *Cryptococcus neoformans* | True yeast | Cryptococcal meningitis or pulmonary infection (torulosis) | Worldwide |
| *Histoplasma capsulatum* | Dimorphic | Pulmonary or disseminated histoplasmosis | USA mainly |
| *Blastomyces dermatidis* | Dimorphic | North American blastomycosis | North America |
| *Sporotrichum schenckii* | Dimorphic | Sporotrichosis | USA and France mainly |
| *Coccidioides immitis* | Dimorphic (closest)* | Coccidioidomycosis (San Joaquin Valley fever) | USA—south western |

* 'Sporangia' in tissues, filamentous at 22 °C.
produced by *Aspergillus flavus* in cereals in less developed countries and ingestion of these toxins may cause liver damage possibly also predisposing to the development of hepatoma.

**PATHOGENESIS: FACTORS AFFECTING THE 'VIRULENCE' AND SPREAD OF MICROBES**

*Pathogenicity*

Microbes can be classified into ‘pathogens’, ‘commensals’ which are found in the normal body flora and ‘saprophytes’ which are found in environmental sites such as soil or plants. However, such a classification is of limited value since there are many examples of ‘commensals’, such as *Escherichia coli*, *Staph. saprophyticus* or *Streptococcus viridans* or saprophytes, such as *Mycobacterium kansasii* or *Legionella pneumophila* which may cause disease in patients under certain circumstances. The ‘pathogenicity’ of a microbe depends on host as well as on microbial factors and microbes can be usefully classified into ‘conventional pathogens’, ‘conditional pathogens’ and ‘opportunist pathogens’ (see Chapter 25).

Host factors include the age of the patient, genetic factors, general host defences and local host defences against infection (see Chapter 6 and Immunodeficiency, in Chapter 8).

‘Koch’s postulates’ have sometimes been useful for establishing the pathogenic relationship between a microbe and a disease. These postulates include the following: (1) the particular microbe is always associated with a given disease (this microbe may be either the cause or an incidental result of the disease); (2) the microbe may be isolated in the laboratory from specimens from a patient with the disease; (3) it is possible to produce a similar disease in animals by inoculation of the microbe into animals. *Mycobacterium tuberculosis* causing tuberculosis may be taken as an example where these three postulates may be fulfilled, but there are many other examples where complete fulfilment of these postulates does not occur, as with *Treponema pallidum* and syphilis, Epstein–Barr virus and glandular fever, *Chlamydia trachomatis* and non-specific urethritis.

**Factors affecting ‘Virulence’**

There is a lot of variation between strains of the same microbial species or between different species, in the ‘virulence’ of the microbe when considering the likelihood of disease being produced in a given ‘host’. An experimental measure of the ‘virulence’ can sometimes be obtained by estimating the LD$_{50}$ (lethal dose) which is the dose of organisms required to kill 50% of the animal population inoculated with the particular microbe. The more virulent the strain the lower is the LD$_{50}$.

The main known factors that affect virulence are concerned with pathogenicity, such as toxins and capsules in bacteria, examples of which are included in Table 1.5. In recent years, there has been an increased interest in bacterial adhesiveness factors, such as the pili of gonococci or of *E. coli* strains that cause urinary tract infections (see Chapter 19). It has also become apparent that the ‘virulence’ of bacterial strains may also depend on the presence of transmissible genes contained in plasmids or mediated by bacteriophage. The adhesiveness to
Table 1.5. Factors affecting 'virulence' of bacteria—some examples

| Virulence factor | Bacterial examples | Comment |
|------------------|--------------------|---------|
| **I. Toxins**    |                    |         |
| i. ‘Classic exotoxins’ | Gram-positive bacteria mainly, e.g. |
|                  | Clostridium tetani toxin | i. Exotoxins are highly toxic polypeptides excreted by living bacteria (micrograms kill animals) |
|                  | Clostridium perfringens (welchii) toxin | They act at specific target sites, e.g. CNS, heart |
|                  | Clostridium botulinum toxin | ii. They are highly antigenic (exception is Cl. tetani toxin) |
|                  | Corynebacterium diphtheriae toxin | iii. Converted to antigenic non-toxic toxoids by formalin |
|                  | ii. Other exotoxins | iv. The toxin is neutralized by anti-toxin |
|                  | Produced by Strep. pyogenes strains causing scarlet fever | v. The toxoids are often destroyed by heat |
|                  | Streptococcal erythro- | Heat stable |
|                  | genic toxin | |
|                  | Staph. aureus enterotoxin | Consists of three factors which combined in a complex cause oedema, haemorrhage and collapse |
|                  | Bacillus anthracis toxic complex | These enterotoxins may produce diarrhoea after stimulating epithelial adenylate cyclase |
|                  | Vibrio cholerae enterotoxin | |
|                  | Escherichia coli enterotoxin | |
|                  | Shigella dysenteriae entero-/neurotoxins | |
| ii. ‘Classic endotoxins’ | Gram-negative bacteria mainly, e.g. |
|                  | Salmonella typhi | i. Endotoxins are lipopolysaccharide (LPS) molecules in the outer layer of Gram-negative cell walls (released when organisms disintegrate). Lipid A is the main toxic component (hundreds of micrograms kill animals). They act nonspecifically on RES cells stimulating release of mediators affecting vascular permeability and release of prostaglandins that may cause fever |
|                  | Neisseria meningitidis | |
|                  | Escherichia coli | |
|                  | Pseudomonas aeruginosa | |
|                  | ii. The sugar chains present in the polysaccharide core of LPS confer ‘O’ antigen specificity and also affect virulence (‘ROUGH’ strains instead of ‘SMOOTH’ strains when the projecting sugar chains are shortened and the Gram-negative bacteria become less virulent) |
|                  | iii. Not converted to toxoids by formalin |
### Table 1.5 (cont.)

| Virulence factor | Bacterial examples | Comment |
|------------------|--------------------|---------|
| iv. Enzymes      |                    |         |
| e.g.             | Staph. aureus      |         |
|                  | coagulase          |         |
|                  | Strep. pyogenes    |         |
|                  | streptolysins      |         |
|                  |                    | Role of enzymes in man often unclear |
|                  |                    | Coagulase may contribute to 'wallowing off' of staphylococcal lesions |
|                  |                    | Streptolysins can induce lysosomal discharge and kill polymorphs and inhibit chemotaxis |

### II. Capsules and other surface antiphagocytic factors

e.g. Capsule of

- Haemophilus influenzae
- Pittman type b (polysaccharide)
- Capsule of Strep. pneumoniae (polysaccharide)
- Capsule of Bacillus anthracis (polyglutamic acid)
- K antigen of Escherichia coli (polysaccharide)
- Vi antigen of Salm. typhi
- 'M protein' of Strep. pyogenes
- Protein A of Staph. aureus

These factors may contribute to the 'invasiveness' of some virulent bacteria by rendering the bacteria relatively resistant to either phagocytosis or killing within polymorphs or macrophages. Certain capsulated bacteria may multiply in macrophages and be disseminated throughout the body as a result, e.g. Salm. typhi bacilli with Vi antigen.

If specific antibody (opsonins) has developed to the capsule or surface component the antiphagocytic effect may be reduced.

Blocks phagocytosis of opsonized pathogenic strains of Staph. aureus possibly by interfering with attachment of Fc portions of IgG opsonins to surface of polymorphs.

---

the ileal mucosa of an *E. coli* strain that produces enteritis in pigs is dependent on the presence of the K88 capsular antigen, a factor which is plasmid mediated. Enterotoxin production by this *E. coli* strain is also dependent on the presence of
the appropriate plasmid. In man, the toxins produced by *Corynebacterium diphtheriae* and the erythrogenic toxin produced by *Strep. pyogenes* strains in scarlet fever patients are dependent on genes mediated by temperate phages. The fact that particular microbes appear to be more or less virulent at different times, might be due in part to the presence or absence of these types of transmissible genes. Scarlet fever is much less frequent than 50 years ago although streptococcal sore throats are still common. Skin sepsis due to *Staph. aureus* strains in hospital maternity units appears to be much less serious than in the 1950s. The microbial and the other factors that affect the virulence, and the spread of pathogens are often unclear (see Williams, 1976).

### Factors affecting Spread

Epidemiological factors affecting the 'host' are relevant to the spread of microbes including the numbers of susceptible individuals in a geographically defined area, the proximity of the individuals to each other and to the source of infection, and the presence of other factors necessary for the transmission of infection, such as the correct climate or season, the presence of an essential arthropod vector, etc. These and other factors are discussed where relevant in the subsequent chapters where sporadic, endemic or epidemic infections are described.

Microbial factors that affect the spread depend partly on the 'virulence' of the microbe and partly on the ability of the microbe to survive or multiply in a given inanimate environment ('fomites' such as bedclothes, 'vehicles' such as milk or water) or on the hands of patients or hospital staff or in animals/arthropods. Above all, the microbe must have the ability to initiate an infection in a patient in as low a dose as possible, have an effective portal of entry for establishing infection, as well as a method of exit from the body where it can be shed in large numbers for as long as possible. 'Carrier' states clearly aid the transmission of bacteria. Gram-positive bacteria survive reasonably well in 'dry' environments while Gram-negative bacteria and some spirochaetes survive best in moist situations.

Microbes are either transmitted horizontally, i.e. between individuals of the same generation (such as the plague bacillus) or vertically, i.e. between individuals of different generations (such as congenital rubella from mother to infant). Hepatitis B is one example of an infection that is vertically transmitted between many millions of people in the less developed world.

Infection is either endogenous, from the patient's own flora, or exogenous, from a source outside such as another patient or person, an animal, a 'vehicle' or 'fomite'. Modes of transmission of microbes include: (1) direct contact, such as with *Neisseria gonorrhoeae*; (2) ingestion, such as with *Vibrio cholerae*; (3) inoculation, such as with a 'sharps' injury transmitting hepatitis B, mosquito bite transmitting malaria or dog bite transmitting rabies; (4) inhalation, such as with measles virus, rhinoviruses or *Mycobacterium tuberculosis*. Numerous diseases are transmitted by the airborne route either by sprays of infected droplets or secretions (by coughing, sneezing or spitting) which contaminate clothing, hands, handkerchiefs (such as with rhinoviruses causing common colds) or by respiratory droplet nuclei (such as with measles virus). The droplet nuclei (1–10 μm in diameter) result from the evaporation of large droplets and may travel long distances as they become suspended in the air.
Appendix: Basic characteristics of some important bacterial pathogens

Notes on some basic characteristics of bacteria that can be Gram-stained are included in this appendix (see also Table 25.1, in Chapter 25; typing methods are also referred to in Chapter 25).

**Gram-positive Bacteria**

**Staphylococci**

- **Microscopy:** Gram-positive cocci mainly in clusters
- **Culture:** white, cream or golden yellow 0.5–1.5 mm colonies on blood agar after overnight aerobic incubation

**Differential test**

**COAGULASE TEST**

This is the main differential test. Coagulase is an enzyme which converts fibrinogen in plasma to fibrin, thus producing clumping when coagulase-positive staphylococci are mixed with plasma in a slide coagulase test and clot formation in a tube coagulase test.

- Coagulase-positive staphylococci indicates *Staph. aureus*
- Coagulase-negative staphylococci indicates *Staph. albus (Staph. epidermidis)* or *Staph. saprophyticus*

**Staphylococcus aureus**

Found in the nose of 10–30% of normal people, but only occasionally on healthy skin, it is a common cause of infection in the community and in hospital. Most infections are sporadic but occasional outbreaks occur.

**DISEASES INCLUDE:**

1. Skin infections including boils, carbuncles, breast abscess, surgical wound infection, neonatal skin sepsis and rare toxic complications such as toxic epidermal necrolysis and toxic shock syndrome.
2. Deep tissue infections including pneumonia, osteomyelitis, septic arthritis, endocarditis.
3. Septicaemia and complications of septicaemia including disseminated intravascular coagulation, endocarditis and metastatic abscesses.
4. Food poisoning; staphylococcal enterocolitis.

**PREDISPOSING HOST FACTORS FOR STAPHYLOCOCCAL INFECTIONS INCLUDE:**

Diabetes mellitus, neutropenia, hypogammaglobulinaemia, and rare phagocyte defects as in chronic granulomatous disease.
ANTIBIOTICS
Penicillin, cloxacillin, erythromycin, lincomycin, fusidic acid and vancomycin are examples of narrow spectrum anti-staphylococcal antibiotics.

Greater than 90%, hospital strains and 60%, community strains are resistant to penicillin because of penicillinase production and these strains would also be resistant to other penicillins, such as ampicillin, but are usually sensitive to cloxacillin (or flucloxacillin).

Multiple antibiotic resistance to two or more different antibiotics may occur, especially in hospital, e.g. *Staph. aureus* resistant to penicillin, tetracycline, erythromycin, lincomycin and fusidic acid. Antibiotic resistance is often plasmid mediated and the spread of plasmids between different strains of *Staph. aureus* is facilitated by transducing phages. Increasing problems with epidemic strains of *Staph. aureus* resistant to methicillin (cloxacillin) and other antibiotics (MRSA) have occurred in hospitals throughout the world. Serious infections due to MRSA are best treated with intravenous vancomycin.

*Staphylococcus epidermidis (Staph. albus), and 'Staph. saprophyticus'*
Found normally in the nose or skin flora of healthy people.

DISEASES INCLUDE:
Urinary tract infections, endocarditis after heart surgery or in the elderly, shunt infections in infants with hydrocephalus and infections of hip joint prostheses.

ANTIBIOTICS
Antibiotic sensitivity patterns of different strains of *Staph. epidermidis* vary greatly, many strains being resistant to several antibiotics often including methicillin (cloxacillin), and serious infections may be treated with vancomycin.

**Streptococci**

Microscopy: Gram-positive coccii, either in chains as with β-haemolytic streptococci or viridans streptococci, or as diplococci, as with pneumococci.

Capsules of *Strep. pneumoniae* may occasionally be seen in Gram films and in special stained smears for capsules.

Culture: Most streptococcal colonies on blood agar are apparent after 24–48 hours incubation aerobically. Some micro-aerophilic or anaerobic streptococci require up to 5 days incubation anaerobically before colonies are seen.

Some pneumococcal strains, *Strep. milleri* strains and some viridans streptococci, such as *Strep. mutans*, grow best when 5–10% carbon dioxide is added to the atmosphere for incubation.
**Differential tests**

CLASSIFICATION ACCORDING TO HAEMOLYSIS ON BLOOD AGAR

- **Alpha (α) haemolysis**
  - green colour around each colony due to altered haemoglobin, e.g. *Strep. viridans*

- **Beta (β) haemolysis**
  - complete lysis of red cells around each colony. This is often most obvious on the anaerobic plate, e.g. *Strep. pyogenes*

- **Gamma (γ) haemolysis**
  - non-haemolytic colonies, e.g. *Strep. faecalis*

1. **Alpha-haemolytic streptococci**
   - *Strep. pneumoniae* and viridans streptococci. However, some viridans streptococci may also appear as non-haemolytic colonies. Pneumococcal colonies often classically have a ‘draughtsman’ appearance.

   **Optochin (diethylhydrocuprein) and bile solubility tests**
   - **Optochin disc test**
     - pneumococci, but not viridans streptococci, show a zone of inhibition around optochin
   - **Bile solubility test**
     - pneumococci, but not viridans streptococci, are soluble in a bile salt suspension

**Biochemical tests for viridans streptococci**

Viridans streptococci can be identified further by biochemical tests, such as the production of dextran from sucrose, into species including *Strep. mitior, Strep. sanguis, Strep. mutans, Strep. salivarius* and *Strep. milleri*.

2. **Beta-haemolytic streptococci**

   These are differentiated mainly by Lancefield grouping.

   **Lancefield grouping and the ‘bacitracin test’**

   Polysaccharide antigen is extracted from the streptococcal cell walls for Lancefield grouping and the specific group antigen is identified using known antisera, such as in a precipitin test or in a coagglutination commercially available latex slide test.

   The Lancefield grouping test may be used to identify some other streptococci which are not necessarily beta-haemolytic. In practice this test is most frequently carried out with beta-haemolytic streptococci. Important examples of different streptococci that can be put into Lancefield groupings include:
   - **Lancefield group A**—synonymous with ‘*Strep. pyogenes***
     - Greater than 90% *Strep. pyogenes* strains are sensitive to a bacitracin identification disc. This bacitracin test is often used to presumptively identify beta-haemolytic streptococci on blood agar as *Strep. pyogenes*, especially in cultures of throat swabs. This test is not entirely reliable as...
other streptococcal species may sometimes be sensitive to bacitracin. Also a few strains of *Strep. pyogenes* may appear with reduced sensitivity to bacitracin.

The group A Lancefield antigen is distinct (polysaccharide) from the other cell wall antigens in *Strep. pyogenes* which are used to type strains in outbreaks such as the M 'virulence' protein and T protein antigens *(see Typing, p. 604).*

b. Lancefield group B—'Strep. agalactiae'
Some group B streptococcal strains are only slightly beta-haemolytic. (There are selective media available to assist the isolation of group B streptococci in specimens from a site with mixed flora such as a vaginal swab.)

c. Lancefield group C and Lancefield group G streptococci
These beta-haemolytic streptococci are frequently isolated from normal (or infected) throat swabs or infected skin sites.

d. Lancefield group D
The main examples include *Strep. faecalis* and *Strep. bovis* although these species usually appear as non-haemolytic colonies on blood agar.

3. NON-HAEMOLYTIC STREPTOCOCCI
These species can be differentiated according to the results obtained with Lancefield grouping, biochemical tests and cultural tests on bile-aesculin agar or MacConkey agar.

*Strep. faecalis* grows on MacConkey agar (magenta colonies) and on bile-aesculin agar (turning this black).

Examples of streptococcal species that frequently appear as non-haemolytic streptococci include *Strep. faecalis, Strep. bovis* and some species of viridans streptococci, such as *Strep. mutans* and *Strep. milleri.*

*Normal flora and streptococcal diseases*
The main streptococcal pathogens and their associated diseases are included in *Table 1.6.*

*Antibiotics*
All *Strep. pyogenes* strains are sensitive to penicillin. Nearly all *Strep. pneumoniae* strains are sensitive to penicillin. The majority of 'Strep. viridans' strains are sensitive to penicillin. *Strep. faecalis* and Lancefield group B streptococci are only moderately sensitive to penicillin.

The great majority of streptococci are sensitive to erythromycin which may be particularly relevant for penicillin-allergic patients.

*Bacillus species*
These include *Bacillus anthracis, Bacillus cereus* and *Bacillus subtilis.*
## Table 1.6. Streptococci and disease

| Streptococcus                      | Usual haemolysis | Normal site                                              | Main associated diseases include                                      |
|-----------------------------------|------------------|----------------------------------------------------------|-----------------------------------------------------------------------|
| *Strep. pneumoniae*               | ß                | Throat and nose (up to 70% population)                    | Otitis media, sinusitis, mastoiditis, pneumonia, meningitis, brain abscess |
| ‘Strep. viridans’ e.g. *Strep. mitior, Strep. sanguis, Strep. mutans* and *Strep. milleri* | ß                | Mouth                                                   | Bacterial endocarditis                                                |
| *Strep. pyogenes* (Lancefield group A) | ß                | Throat (up to about 5% population)                       | Sore throat, scarlet fever, otitis media                              |
|                                   |                  |                                                         | Later complications—rheumatic fever, acute glomerulonephritis          |
|                                   |                  |                                                         | Skin infections including erysipelas, impetigo, infected traumatic or  |
|                                   |                  |                                                         | eczematous lesions                                                   |
|                                   |                  |                                                         | Wound infections and puerperal sepsis                                 |
|                                   |                  |                                                         | Septicaemia (e.g. complicating cellulitis)                            |
| Lancefield group B streptococci   | β                | Perineal skin lower vagina (5–30% women)                 | Neonatal septicaemia and meningitis                                  |
| Lancefield group C or G streptococci | β                | Throat                                                  | Sore throat (very occasionally), skin infections, septicaemia          |
| *Strep. faecalis*                 | Non              | Intestine                                               | Urinary tract infection, bacterial endocarditis                        |
| *Strep. bovis*                    | Non              | Intestine                                               | Bacterial endocarditis                                                |
| Micro-aerophilic or anaerobic streptococci | Non              | Skin, throat or lower vagina                            | Meleney’s synergistic gangrene (together with *Staph. aureus*)        |
|                                   |                  |                                                         | Cellulitis, such as skin or female genital tract infection             |
|                                   |                  |                                                         | Cerebral abscess (often mixed with other organisms)                   |

### Microscopy:

*Bacillus anthracis* is typically seen as large square-ended Gram-positive bacilli, sometimes in long chains. (Other aerobic-spore-bearing bacilli including *Bacillus subtilis* may appear as Gram-variable or Gram-negative bacilli.)
Capsules of \( B. \) anthracis are stained purple in McFadyean’s reaction with a polychrome methylene blue stain; other \( Bacillus \) species do not show capsules.

\( B. \) anthracis spores are not apparent in spore stains of clinical specimens from infected patients but may be present in environmental specimens.

**Culture:**

\( B. \) anthracis colonies are seen after overnight culture on blood or nutrient agar as rough opaque colonies with edges resembling loose curls of hairs. Other \( Bacillus \) species do not have colonies with this type of edge. (\( B. \) subtilis, \( B. \) cereus and other bacilli are common blood culture contaminants which may sometimes be confused with ‘coliforms’ when colonies appear on MacConkey’s medium.)

\( B. \) anthracis gives a characteristic inverted fir tree growth in gelatin.

**Differentiation of Bacillus species by animal inoculation tests**

\( B. \) anthracis, but not the other \( Bacillus \) species, is pathogenic to mice and guinea-pigs. This test is too dangerous to use in the average hospital animal house. Infected animals may disseminate anthrax spores which could survive for many years in the environment.

**Sources and diseases**

\( B. \) anthracis can infect many different animal species including sheep and the anthrax spores may remain viable in animal products or in the soil for a long period. \( B. \) cereus and \( B. \) subtilis are found in the soil, dust and air and \( B. \) cereus may contaminate food such as boiled rice.

\( B. \) anthracis is the cause of anthrax in man and animals (see Chapter 16). \( B. \) cereus is one cause of food poisoning (see Chapter 14). \( B. \) subtilis is nearly always only a contaminant when noticed in cultures. However, it may rarely cause bacteraemia in patients on haemodialysis when the dialysis machines are contaminated or when an intravenous infusion has become contaminated.

**Antibiotics**

\( B. \) anthracis is characteristically sensitive to penicillin.

**Corynebacterium species**

These include \( Corynebacterium \) diphtheriae, \( Corynebacterium \) ulcerans, and ‘diphtheroid species’ including \( Corynebacterium \) xerosis and \( Corynebacterium \) hoffmani.
Microscopy: The Gram-positive bacilli of *C. diphtheriae* are slightly curved and characteristically appear like 'Chinese characters' whereas the diphtheroid bacilli are often seen as palisade rows of bacilli.

Metachromatic granules ('Volutin granules') are sometimes seen in an Albert's stain of diphtheria bacilli. However, these granules do not necessarily indicate that the bacilli are *C. diphtheriae*, nor do they reliably indicate that a diphtheria strain is toxigenic.

Culture: Many 'diphtheroids' can easily be distinguished from *C. diphtheriae* according to the colonial appearances on tellurite media (such as Hoyle's or Downie's medium) and on blood agar. *C. diphtheriae* appears characteristically as grey-black colonies on tellurite. However, a few 'diphtheroid' strains may be difficult to differentiate in this way and any suspicious colonies require further tests.

Loeffler’s serum agar slope is also used for the isolation of *C. diphtheriae* and is useful for providing a suitable culture for toxigenicity tests.

**Differential tests**

Hiss’s serum water sugar fermentation tests are inoculated and the pattern of results helps to identify the *Corynebacterium* species. Characteristically *C. diphtheriae* ferments glucose and maltose, rarely sucrose. The *C. diphtheriae* species can be further differentiated into the subspecies *gravis* (‘daisy head’ classically and ferments starch), *mitis* or *intermedius* but, in practice, this is not important except for epidemiological purposes. *C. ulcerans* can give some biochemical reactions similar to *C. diphtheriae gravis* but the urea slope test reaction is different.

**Toxinogenicity tests**

These urgent tests on suspicious *C. diphtheriae* cultures are performed by an Elek plate or guinea-pig method (see p. 200).

**Normal flora and diseases**

'Diphtheroids' are commonly isolated skin or throat commensals. Rarely, urinary tract infection or bacterial endocarditis affecting a prosthetic heart valve may be caused by these organisms.

*C. diphtheriae* is rarely found in the normal throat flora except during convalescent carriage. Toxigenic strains may cause diphtheria in susceptible individuals.

*C. ulcerans* may cause a severe sore throat and some marked constitutional upset but is rarely associated with the classic toxic complications of diphtheria.
**Antibiotics**

*C. diphtheriae* strains are characteristically sensitive to penicillin and erythromycin.

**Listeria and Erysipelothrix**

*Listeria monocytogenes and Erysipelothrix rhusiopathiae*

**Microscopy:** Short Gram-positive bacilli may occasionally be confused with ‘diphtheroids’.

  Light microscopy of a wet preparation of a peptone water culture of *Listeria monocytogenes*, that has been incubated at room temperature, characteristically shows ‘tumbling motility’.

**Culture:** Small colonies appear on blood agar after overnight incubation at 35°C. Listeria colonies usually show beta-haemolysis but erysipelothrix colonies are usually alpha- or non-haemolytic. *Listeria*, but not *Erysipelothrix*, grows at 4°C.

**Differential tests**

Biochemical tests, as well as cultural characteristics, differentiate *Listeria* from *Erysipelothrix* including tests for aesculin hydrolysis and catalase production.

**Diseases**

*Listeria monocytogenes* is a cause of meningitis and/or septicaemia in neonates and in immunocompromised patients. It is a possible but rare cause of still-birth. 

*Erysipelothrix rhusiopathiae* causes erysipeloid (see p. 382 and p. 518).

**Antibiotics**

*Listeria* and *Erysipelothrix* are both characteristically sensitive to ampicillin (or penicillin).

**Nocardia and Actinomycetes**

These Gram-positive branching filamentous bacilli cause nocardiosis and actinomycosis, respectively (see Chapter 13).

**Clostridial species**

These include *Clostridium perfringens (welchii)*, *septicum*, *oedematiens*, *histolyticum*, *tetani*, *botulinum*, *difficile*.

  The main characteristics of Gram-positive spore-forming anaerobic bacilli are described in Chapter 9.
**Gram-negative Bacteria**

**Neisseria species**

These include *Neisseria gonorrhoeae*, *meningitidis*, *catarrhalis*, *pharyngis* and *lactamis*.

- **Microscopy:** Gram-negative oval diplococci; some are characteristically intracellular when seen in clinical specimens.
- **Culture:** *Neisseria* are fragile and suitable transport of specimens with prompt culture is important (see pp. 232, 462).
  
  Small colonies on blood agar after 24–48 hours' incubation in a moist aerobic atmosphere with 5–10% carbon dioxide added. Larger colonies on chocolate agar.

  Selective media are used for the isolation of *Neisseria gonorrhoeae* from genital tract or rectal specimens (see p. 462). Non-pathogenic *Neisseria* sometimes grow on plain agar.

**Differential tests**

*Neisseria* species are oxidase positive. The species are differentiated according to the results obtained with biochemical and immunological tests.

1. **Biochemical tests**

   Serum sugar agar slopes are used usually for carrying out sugar fermentation tests (not horse serum which contains maltose). Hydrocele fluid can be used instead of serum as a growth factor. Glucose only is characteristically fermented by gonococci. Maltose and glucose are characteristically fermented by meningococci (although a few strains do not ferment maltose). Sucrose or lactose are sometimes fermented by *Neisseria* species which are neither *Neisseria gonorrhoeae* nor *meningitidis*. The media used for sugar fermentation tests needs to be carefully quality controlled.

2. **Immunological tests**

   An immunological test as well as a biochemical test is desirable for the identification of possible gonococcal strains, especially when the isolate is from a female patient or from an unusual site, such as the throat (because atypical strains of other *Neisseria* species, isolated in these circumstances, may occasionally give similar biochemical reactions to those of gonococci). When an isolate shows strong immunofluorescence or gives a positive latex co-agglutination test with a specific anti-gonococcal serum, there is good evidence that the isolate is a gonococcus (but the medicolegal differentiation traditionally depends on the results of biochemical tests).

   Immunological tests are also used in reference centres to serogroup meningococci into one of the three main groups, A, B or C. Most strains in Britain are serogroup B.

**Normal flora and diseases**

*Neisseria meningitidis* is carried in the nasopharynx of 5–30% of the general population, and is one of the 'three primary pathogens' causing bacterial meningitis.
Neisseria gonorrhoeae is the cause of gonorrhoea and ophthalmia neonatorum. It is not found in the normal flora.

**Antibiotics**

All meningococcal strains outside South Africa are sensitive to penicillin. There are some gonococcal strains highly resistant to penicillin (penicillinase producers), but sensitive to spectinomycin and penicillinase-stable cephalosporins, such as cefotaxime. However, well over 90% gonococcal strains in Britain are still relatively sensitive to penicillin.

**Enterobacteria (Coliforms)**

These include scores of different genera and many hundreds of different species. *Escherichia coli, Klebsiella aerogenes, Proteus, Salmonella and Shigella* species are examples (see also Table 1.2).

- **Microscopy:** Gram-negative bacilli—the species are not differentiated by their Gram-stain appearance.
- **Culture:** Good growth on blood agar, MacConkey or cysteine lactose electrolyte deficient (CLED) medium is characteristic after overnight aerobic incubation.
  
  Selective media such as deoxycholate citrate agar (DCA), which suppress the growth of many *E. coli* strains, are used for the isolation of both salmonellae and shigellae from faeces and require up to 48 hours' incubation.
  
  Enrichment liquid media, such as selenite F, are used to increase the yield of salmonella isolations from the faeces. The organisms are subcultured from these media on to selective media, usually after overnight incubation.

**Differential tests**

1. **Lactose fermentation**

   *Escherichia coli, Klebsiella* or other coliforms which are characteristically lactose fermenters appear as pink colonies after overnight culture on MacConkey or CLED media. However, some late lactose fermenting strains of these species may appear as 'non-lactose fermenting' colonies on MacConkey agar. *Salmonella* and *Shigella* species characteristically appear as 'non-lactose fermenters' on MacConkey or DCA media (but *Shigella sonnei* is a late lactose fermenter and may appear slightly pink on MacConkey after 24–48 hours' incubation).

2. **Motility and other biochemical tests**

   A 'hanging drop' of an overnight peptone water culture may be examined by wet microscopy to see if the organism is motile or non-motile. *E. coli* and salmonellae are characteristically motile (they are flagellated coliforms) whereas klebsiellae and shigellae are characteristically non-motile. Semi-solid agar methods are available (e.g. 'Craigie tube') for testing for the motility of a possible salmonella or shigella isolate, which are safer than the hanging drop method.
Biochemical tests, in addition to lactose fermentation, include urea, glucose, mannite, sucrose, indole, citrate, hydrogen sulphide production and decarboxylases for apparent 'non-lactose fermenters' and indole, citrate and inositol for 'lactose fermenters'.

Commercial kits are available to assist this identification process which must be carried out on pure cultures. For the identification of 'difficult' organisms, a computer analysis of the results may be useful at a reference centre.

A few simple characteristic examples of the results of biochemical tests include:

- **Proteus species** urease positive, lactose negative
- **Salmonella species** glucose fermented, mannite fermented, lactose negative, urea negative, sucrose negative
- **Shigella species** glucose fermented
  - mannite fermented—mannitol positive
  - shigellae (e.g. *Sh. sonnei*)
  - urea negative, sucrose usually negative
  - mannite negative—mannitol negative
  - shigellae

- **E. coli** indole positive, lactose positive, citrate negative
- **Serratia marcescens** DNAase positive.

There are also classic 'IMVIC' tests for lactose fermenters isolated from possibly faecally contaminated water supplies—indole, methyl red, Voges-Proskauer, inositol and citrate. These biochemical tests may be performed at 44°C to recognize *E. coli* type I from a possible human faecal source (e.g. from polluted water).

3. **Immunological tests**

Suspensions of suspected pathogenic faecal coliforms can be tested against known specific antisera in slide or tube agglutination tests. Specific 'O' (somatic antigen) antisera are used for the identification of isolates of salmonella, shigellae, and enteropathogenic strains of *E. coli*. Specific 'H' (flagellar antigen) antisera are mainly used to identify *Salmonella* species. A salmonella culture on a nutrient agar is often used for agglutination tests but it may be in a 'non-specific H phase'. To convert the *Salmonella* to a 'specific H phase', a 'phase switch' may be necessary using a Craigie tube method.

**Normal flora**

*E. coli* is the most common and most numerous aerobic Gram-negative species in the normal faecal flora. Other coliforms are also often present mixed with the *E. coli*, including *Proteus*, *Klebsiella* or *Citrobacter* species to mention just a few possibilities.

*Salmonella* and *Shigella* species are not found in the normal flora although they may be found in the faeces of healthy convalescent carriers (or permanently in a biliary tract carrier of *Salm. typhi*).
Diseases

‘Endogenous’ infections are most common with lactose fermenting coliforms, such as *E. coli* and certain non-lactose fermenting coliforms, such as *Proteus* species, including urinary tract infections, wound infections, abdominal sepsis and Gram-negative septicaemia.

‘Exogenous’ infections may also occur with the same organisms as those endogenous infections. Cross-infection or environmental infection in hospital may occur (see Chapter 25). Infections of the gastro-intestinal tract are also exogenous, due to salmonellae, shigellae, enteropathogenic *E. coli*, etc. (see Chapter 14).

Antibiotics

There is such an enormous variation in the antibiotic susceptibilities of different coliform strains that they can only be predicted to a limited extent.

1. *Outside hospital*

Many coliforms are sensitive to ampicillin (although *Klebsiella* is an exception), provided no recent antibiotics have been given to the patient. The coliforms are usually sensitive to trimethoprim.

2. *In hospital*

Coliforms are frequently resistant to ampicillin and are often also resistant to other agents, including sulphonamides, tetracycline and streptomycin. The antibiotic resistance patterns vary between different hospitals and between different places in the same hospital, as well as at different times. The patterns of antibiotic resistance depend greatly on the amounts of particular antibiotics used in a given hospital area, the prevalence of particular R factors which carry genes for multiple antibiotic resistance and the frequency of cross-infection in the hospital. In most hospitals in Britain, the great majority of coliforms are still sensitive to gentamicin and to new cephalosporins such as cefuroxime. However, outbreaks of gentamicin-resistant coliform infections occasionally occur.

Pseudomonas species

These include *Pseudomonas aeruginosa*, *Pseudomonas cepacia* and other *Pseudomonas* species.

- **Microscopy:** Gram-negative bacilli, indistinguishable from the ‘coliforms’ above on Gram-stain.
- **Culture:** *Pseudomonas* species are strictly aerobic. Good growth only occurs after overnight incubation in an aerobic atmosphere on a blood or nutrient agar plate in contrast to ‘coliform’ species which can grow well either aerobically or anaerobically as they are facultative anaerobes.

  *Pseudomonas aeruginosa* also grows well on a selective agar containing the disinfectant ‘cetrimide’ and in many solutions.

- **Oxidase test:** *Pseudomonas* species are, characteristically, strongly oxidase positive in contrast to ‘coliform’ species which are oxidase negative.
**Differential tests**

*Pseudomonas aeruginosa* (pyocyanea) produces a green ‘pyocyanin’ pigment on magnesium ion containing media whereas other *Pseudomonas* species do not produce this pigment. It metabolizes glucose by oxidation rather than by fermentation and this can be shown by a ‘Hugh and Leifson’ test. The other *Pseudomonas* species can be differentiated according to the results of biochemical tests (using ammonium salt sugars).

**Normal flora and sources**

*Pseudomonas aeruginosa* occurs infrequently in the faecal flora of patients outside hospital. Hospital patients receiving broad-spectrum antibiotics, such as oral cephalosporins, are frequently colonized by *Pseudomonas aeruginosa* in the lower intestinal tract.

Most *Pseudomonas* species may be isolated from moist environmental sites in the hospital including contaminated suction apparatus, contaminated disinfectants, respiratory ventilators and humidifiers. Bottles containing sterile distilled water or other solutions may become quickly contaminated by Gram-negative bacilli, including *Pseudomonas* species, once the bottles are opened.

**Diseases**

Endogenous or exogenous pseudomonas infections (cross-infection or environmental infection) include chronic urinary tract infections, wound infections, chronic osteomyelitis, chronic otitis externa, eye infections (rare), and various serious opportunistic infections including pneumonia and septicaemia.

**Antibiotics**

*Pseudomonas aeruginosa* is resistant to many antibiotics including ampicillin, sulphonamides, trimethoprim, tetracycline, cephaloridine and many cephalosporins, streptomycin and kanamycin. Nearly all *Pseudomonas aeruginosa* strains are sensitive to polymyxin but this antibiotic is mainly suitable for treating only superficial infections topically. Most strains are sensitive to the aminoglycosides, gentamicin or netilmicin, which are often valuable for systemic treatment. Many strains are sensitive to carbenicillin, ticarcillin, piperacillin or azlocillin and these anti-pseudomonas penicillins are usually used systemically together with the above aminoglycosides. There is a lot of variation in the sensitivity of different strains to these penicillins depending on whether the strains produce particular penicillinases. Most strains are sensitive to ceftazidime, ciprofloxacin and imipenem.

**Vibrios**

These include *Vibrio cholerae*, *Vibrio parahaemolyticus* and *Campylobacter* species (previously known as *Vibrio foetus*), such as *Campylobacter jejuni*.

**Microscopy:** Vibrios characteristically appear as curved Gram-negative bacilli, like ‘comma’ bacilli, but they may also be indistinguishable from ‘coliforms’ in a Gram-stain.
Campylobacters are characteristically seen as spiral or small ‘S’-shaped Gram-negative bacilli in a Gram-stain.
Vibrios are typically motile when suspensions are examined by ‘wet microscopy’.

**Culture:**
Thiosulphate–citrate–bile salt–sucrose agar (TCBS) medium is the selective medium used for the isolation of *Vibrio cholerae* and *Vibrio parahaemolyticus* from faeces. *V. cholerae* and *V. parahaemolyticus* usually produce large yellow or green colonies, respectively, on TCBS medium after 24 hours incubation in an aerobic atmosphere.

An alkaline peptone water enrichment culture is used in addition to TCBS selective medium for the isolation of *V. cholerae*.

*Campylobacter* species grow well after 24–48 hours incubation on a selective blood agar medium containing polymyxin, vancomycin and trimethoprim. The plates have to be incubated in a micro-aerophilic atmosphere with added carbon dioxide, preferably at 40–42 °C. When this medium is used to isolate *Campylobacter* species from the faeces of a patient, a presumptive diagnosis of *Campylobacter* is often possible by seeing ‘S’-shaped or slim curved Gram-negative bacilli in a Gram-stain of the characteristic moist-looking, oxidase-positive colonies.

**Differential tests for V. cholerae**
Suspicious yellow colonies on TCBS medium are further identified by Gram-stain, oxidase test, subculture on to nutrient or blood agar for definitive immunological tests and rapid slide agglutination tests with specific *V. cholerae* anti-serum. A presumptive identification is made by the laboratory which urgently sends the culture to a reference laboratory for confirmatory tests including phage typing (with the Mukerjee phage). The results of phage and polymyxin sensitivity tests, haemolysis and other tests in the reference laboratory can also differentiate between ‘El Tor’ and the ‘classic’ biotypes of *V. cholerae*. Nearly all the patients with cholera seen in Europe, the Middle East and Africa have been infected by the ‘El Tor’ *V. cholerae*.

**Diseases**
*V. cholerae* causes cholera and the strains may be carried during convalescence.
*V. parahaemolyticus* is an uncommon cause of food poisoning in Britain.
*Campylobacter jejuni* is a common cause of gastro-enteritis and food poisoning.
*Campylobacter pylori* is associated with some types of peptic ulceration (see Chapter 14).

**Antibiotics**
*V. cholerae* (El Tor) is usually sensitive to tetracycline but the incidence of tetracycline-resistant strains is increasing. Antibiotics are of secondary importance to fluid and electrolyte replacement (see p. 356).
*Campylobacter jejuni* is nearly always sensitive to erythromycin.
**Parvobacteria**

These include: *Haemophilus influenzae* and other *Haemophilus* species; *Bordetella pertussis* and *parapertussis*; *Pasteurella multocida* (septica); *Yersinia pseudotuberculosis*, *Yersinia enterocolitica* and other *Yersinia* species; *Brucella abortus* and other *Brucella* species; *Francisella tularensis*; *Pseudomonas mallei*.

**Microscopy:** The parvobacteria are short Gram-negative bacilli (coccobacilli). Many such as *Haemophilus* species are pleomorphic. Some bacilli, such as *Pasteurella multocida* may show bipolar staining. Occasionally, the capsules of capsulated strains of *Haemophilus influenzae* may be seen, e.g. Pittman type b strain, in stained smears or by immunofluorescent techniques using specific anti-capsular antibody.

**Culture:** Parvobacteria are relatively fragile and specimens with these organisms need to be promptly cultured and preferably inoculated directly on to media for the best culture results. If delays are inevitable, appropriate transport media may be used for certain species such as *Bordetella* (see p. 196).

Most parvobacteria species appear as small colonies on fresh blood agar after 24–48 hours’ incubation in an aerobic moist atmosphere with 5–10% added carbon dioxide. A few species will not grow on blood agar, such as *Bordetella pertussis*. Special enriched and selective media such as Bordet-Gengou, Lacey’s medium or pertussis charcoal agar are needed for culture of *Bordetella*. Dorset’s egg medium is required for culture of *Francisella tularensis*.

*Haemophilus* species grow better on chocolate agar than on blood agar; moderately large colonies are apparent on chocolate agar after overnight incubation.

**Differential tests**

1. *Haemophilus* species

The *Haemophilus* species can be identified according to the results of ‘satellitism’ tests. *Haemophilus influenzae* will not grow around a disc containing factor X (haemin) or factor V (coenzyme NAD) alone, but will around a combined X plus V disc on plain agar. On blood agar, *Haemophilus influenzae* shows improved satellite growth around *Staph. aureus* colonies due to the release of factor V from the staphylococci. (*Haemophilus parainfluenzae* does not require factor X but does require factor V.)

Haemolysis is another cultural factor that is used for identifying less pathogenic *Haemophilus* species, such as *Haemophilus haemolyticus*, which may be found in the normal throat flora.

Capsulated strains of *Haemophilus influenzae* often grow well with a green sheen on Levinthal's agar. The capsules can be Pittman typed using specific anti-capsular sera (in a Quelling type reaction). The usual capsular type *H. influenzae* infecting infants is Pittman type b. This capsular antigen may sometimes also be detected using an immunoprecipitation test.
2. Differentiation from coliforms
Most parvobacteria species will not grow well (if at all) on MacConkey’s agar after overnight incubation, in contrast to ‘coliforms’ and most *Pseudomonas* species. Most of the parvobacteria will not grow in peptone water sugars unlike coliforms, an important exception being *Pasteurella multocida*. Some parvobacteria will not grow anaerobically on blood agar after overnight incubation unlike coliforms.

3. *Yersinia*, *Pasteurella* and *Bordetella* species
A range of cultural tests on MacConkey agar, blood agar, enriched and selective media combined with biochemical tests, motility and haemolysis tests help to identify a particular *Yersinia* or *Pasteurella* species. Immunological tests using slide or tube agglutinations of suspensions of the organisms against known antisera are also required for *Yersinia* and *Bordetella* species.

**Normal flora**
*Haemophilus* species are frequently found in the normal throat flora and occasionally in the nose flora (especially of infants). *Pasteurella multocida* is also occasionally found in the normal upper respiratory tract flora, especially in individuals who may have contact with rodents (because they work in an animal house or have rodent pets).

**Diseases**
Parvobacteria causing diseases are included in Table 1.7. The most common parvobacteria infections in Britain include those due to *Haemophilus influenzae*, *Bordetella pertussis* and *Pasteurella multocida*. Other parvobacteria infections are very uncommon.

**Antibiotics**
Some parvobacteria species are relatively sensitive to penicillin in contrast to coliforms which are resistant to penicillin, a good example being *Pasteurella multocida*. Many parvobacteria are also sensitive to erythromycin in vitro, such as *Bordetella pertussis*, *Pasteurella multocida*. *Yersinia* and *Brucella* species are characteristically sensitive to tetracyclines.

*Haemophilus influenzae* strains are usually sensitive to ampicillin but the incidence of ampicillin-resistant beta-lactamase-producing strains is increasing and is greater than 10% for Pittman type b capsulated strains in some areas in Britain. Chloramphenicol is nearly always active against *H. influenzae*, including ampicillin-resistant strains and is recommended for treating haemophilus infections such as meningitis or acute epiglottitis. Tetracycline may be used instead of ampicillin for treating infective exacerbations of chronic bronchitis due to *Haemophilus influenzae*.
Table 1.7. Some examples of infections due to parvobacteria

| Parvobacteria                        | Main infections                                                                 |
|--------------------------------------|----------------------------------------------------------------------------------|
| **Haemophilus influenzae**           |                                                                                  |
| i. Capsulated Pitman type b strains  | Infections in children mainly, 3 months to 5 years (up to 12 years may occur):  |
|                                      | a. Respiratory tract infections—pharyngitis, otitis media, sinusitis, acute epiglottitis (rare), pneumonia (very rare) |
|                                      | b. Septicaemia                                                                  |
|                                      | c. Meningitis                                                                    |
|                                      | d. Osteomyelitis and septic arthritis                                            |
|                                      | e. Pericarditis or endocarditis (both rare)                                      |
| ii. Other strains                    | Infections mainly in adults:                                                     |
|                                      | a. Infective exacerbations of chronic bronchitis                                  |
|                                      | b. Chronic sinusitis                                                             |
|                                      | c. Conjunctivitis                                                                |
| **Bordetella pertussis**             | Whooping cough                                                                   |
| **Pasteurella multocida (septica)**  | Wound infections following animal bites: meningitis (rare), septicaemia (rare)    |
| **Yersinia pestis**                  | Plague                                                                           |
| **Yersinia enterocolitica**          | Gastro-enteritis (possible ‘rheumatic fever’-like illness and arthritis possible) |
| **Yersinia pseudo-tuberculosis**     | Mesenteric adenitis (may clinically mimic acute appendicitis)                     |
| **Brucella species**                 | Brucellosis                                                                      |
| **Francisella tularensis**           | Tularaemia                                                                       |
| **Pseudomonas mallei**               | Glanders                                                                         |

**Legionella**

*Legionella pneumophila* and some other *Legionella* species may cause severe pneumonia—Legionnaires’ disease.

**Microscopy:** Gram-negative bacilli in Gram-stains of colonies from culture media; in tissues and clinical specimens the bacilli may stain poorly with an ordinary Gram-stain but better when a prolonged counterstain with carbol fuchsin is used. Gram films made from cultures may show long filamentous forms of the organism. The bacilli are also apparent in silver stains in tissue although they are best seen in tissues by immunofluorescence using specific anti-legionella antisera or by electron microscopy using immunoferritin techniques.

**Culture:** No growth on ordinary media.

Requires 3–5 days’ incubation on special legionella media containing blood, added cysteine and iron salts such as ferric pyrophosphate, at pH 6.9.

Guinea-pig inoculation may be necessary for the isolation of *Legionella* from environmental samples such as water.

**Sources**

Environmental: contaminated water in air conditioning plants, shower mixers, etc., causes infection by inhalation of contaminated air.
**Disease**

Legionnaires' disease ranges from a mild pyrexia of unknown origin (PUO), or mild respiratory symptoms, to severe pneumonia with multisystem complications. It is fatal in about 15% of *Legionella*-infected patients requiring hospital admission for severe pneumonia. Other similar diseases due to ALLO (atypical *Legionella*-like organisms) have been recently reported.

**Antibiotics**

*Legionella* is characteristically sensitive to erythromycin and tetracycline but usually resistant to penicillins and aminoglycosides.

**Anaerobic Gram-negative Bacilli**

Strict non-sporing anaerobic organisms, including Gram-negative bacilli such as *Bacteroides* species, are described in Chapter 9.

**Further Reading**

Christie A. B. (1987) *Infectious Diseases: Epidemiology and Clinical Practice*, 4th ed. Edinburgh, Churchill Livingstone.

Cruickshank R., Duguid J. P., Marmion B. P. et al. (1975) *Medical Microbiology*. Edinburgh, Churchill Livingstone.

Emond R. T. D. (1974) *A Colour Atlas of Infectious Diseases*. London, Wolfe Medical Books.

Jawetz E., Melnick J. C. and Adelberg E. A. (1987) *Review of Medical Microbiology*, 17th ed. Los Altos, California, Lange Medical Publications.

Lambert H. P. (1979) The pathogenesis of diarrhoea of bacterial origin. In: Reeves D. and Geddes A. (ed.) *Recent Advances in Infection*. Edinburgh, Churchill Livingstone.

Mandell G. L., Douglas R. G. and Bennett J. E. (1985) *Principles of Infectious Diseases*, 2nd ed. Chichester, John Wiley.

Mims C. (1982) *The Pathogenesis of Infectious Disease*, 2nd ed. London, Academic Press.

Olds R. J. (1975) *A Colour Atlas of Microbiology*. London, Wolfe Medical Books.

Stokes E. J. and Ridgway G. L. (1987) *Clinical Bacteriology*, 6th ed. London, Edward Arnold.

Stratford B. C. (1977) *An Atlas of Medical Microbiology: Common Human Pathogens*. Oxford, Blackwell Scientific Publications.

Timbury M. (1986) *Notes on Medical Virology*, 8th ed. Edinburgh, Churchill Livingstone.

Williams R. E. O. (1976) The flux of infection. *Proc. R. Soc. Med.* 69, 797–803.

Wilson G. J. and Miles A. A. (1984) *Topley and Wilson's Principles and Practice of Bacteriology, Virology and Immunity*, 7th ed. London, Edward Arnold.

Youmans G.P., Paterson P. Y. and Sommers H. M. (1985) *The Biological and Clinical Basis of Infectious Diseases*, 3rd ed. Philadelphia, Saunders.

* This reference is particularly recommended for further reading by undergraduates.