Bacteriology: Selected Bacteria and Diseases

8

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8.1 *Staphylococcus* Species (Commonly: Staphylococci)

Staphylococci are Gram-positive cocci bacteria, size 5 micrometre, ubiquitously occurring. In the Gram stain, staphylococci are Gram-positive cocci. The cell wall is coloured dark blue because the cell wall is stained with the dark blue stain gentian violet. There are several species of the genus *Staphylococcus*. The most important are:

Staphylococcus aureus
S. aureus is the most frequently isolated pathogen from infection sites in the skin, soft tissue, mucous membranes and internal organs. *S. aureus* is a coagulase-positive *Staphylococcus* and can produce many other antigens and toxins eliciting significant immune response in the human host. *S. aureus* is common pathogen of puerperal mastitis (gestational mastitis), boils and carbuncles. But *S. aureus* is also the primary pathogen of osteomyelitis (bone inflammation) and endocarditis (heart valve inflammation). In food, e.g. not well-cooled vanilla ice cream, *S. aureus* may produce a heat-stable toxin and cause food poisoning with vomiting and diarrhoea. Certain strains of *S. aureus* produce the toxic shock syndrome toxin (TSST-1 or TSST-2), causing a severe illness, toxic shock syndrome and exfoliative dermatitis.

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In the early times, *S. aureus* became resistant to the standard antibiotic against staphylococci, methicillin. It was called then methicillin-resistant *S. aureus* (MRSA). Resistance against methicillin means that MRSA is resistant against nearly all beta-lactam antibiotics. Treatment with beta-lactam antibiotics (penicillins, cephalosporins) is then ineffective. MRSA spread over many hospitals in the United States and the Western world. MRSA is a common cause of hospital infections and can cause epidemics because staphylococci survive well in the environment and are therefore easily transmitted via the items and devices in the environment and hands of the personnel.

Staphylococcus epidermidis
S. epidermidis is a resident on the normal human skin. In contrast to *S. aureus*, *S. epidermidis* is coagulase-negative. However, when transferred to artificial surfaces in the human body (catheters, prostheses), *S. epidermidis* can cause infections of implanted or foreign material. These infections are particularly unpleasant when affecting orthopaedic prostheses. *S. epidermidis* forms a bacterial biofilm consisting of bacterial cells and amorphic material ("matrix") on the material. The treatment of these biofilm-associated infections is nearly not curable without removal of the infected foreign material.

Staphylococcus saprophyticus is a common pathogen of urinary tract infections in women.

There is a large group of other coagulase-negative staphylococci, which can cause infection associated with implanted or accidentally inserted foreign material, similar to *Staphylococcus epidermidis*. There is one species that may be a persistent pathogen like *S. aureus*: *Staphylococcus lugdunensis*.

8.2 *Streptococcus* Species (Commonly: Streptococci)

In the microscope streptococci are looking like a string of pearls. Streptococci have a distinctive property for haemolysing blood in the growth agar:

- α ("alpha")-haemolysis: partial haemolysis of erythrocytes. The *Streptococcus* colonies are surrounded by a green zone.
- β ("beta")-haemolysis: complete haemolysis of the erythrocytes. The *Streptococcus* colonies are surrounded by a yellow zone. Beta-haemolytic streptococci are important pathogens. There are several groups: A, B, C, E and G. Beta-haemolytic streptococci group A and group B are the most important pathogens.

Beta-Haemolytic Streptococci Group A
Classical infections by beta-haemolytic streptococci group A (also called *Streptococcus pyogenes*) are skin infections:

- Wound infections, erysipelas, cellulitis.
- Tonsillitis.

- Scarlet fever, rheumatic fever (hypersensitivity reactions).
- Sepsis (puerperal sepsis, puerperal fever). In puerperal sepsis diagnosis via the vaginal smear with the mother takes place. In neonatal sepsis *Streptococcus pyogenes* can be detected in blood culture, cerebrospinal fluid and urine of new-borns.

Beta-Haemolytic Streptococci Group B

Beta-haemolytic streptococci group B (also called *Streptococcus agalactiae*) can live as normal part of the flora on the mucous membranes (e.g. the vagina–birth canal).

They can cause the following infections:

- Neonatal sepsis and meningitis: Approximately 15% of pregnant women are asymptomatic carriers. In the new-born, it can cause early-onset perinatal infection when the new-born is infected via the birth canal. Neonatal sepsis occurs approximately 3–6 infections per 1000 births. New-borns develop clinical sepsis at 5 to 8 days of age, sometimes associated with meningitis and pneumonia. Left untreated, the mortality rate is as high as at 50%. Alternatively, there can be postnatal late-onset infection via unclean hands or contaminated objects. Here, the incidence is estimated at 2 infections per 1000 births. The symptoms occur from the 2nd week of life.
- Urinary tract infections.
- Pneumonia.
- Atypical erysipelas.

Streptococcus pneumoniae

Streptococcus pneumoniae is also found as a commensal in the normal flora of the respiratory tract. It is the most important causative agent of infection of the upper respiratory tract (infection of the middle ear (otitis media)), pneumonia and sepsis and meningitis (especially in splenectomized patients).

Viridans streptococci

The *Streptococcus viridans* group is an inhomogeneous mixture of alpha-haemolytic streptococci. They are normally part of the oral flora and the intestinal tract. They include the *Streptococcus milleri* group, *Streptococcus mutans*, *Streptococcus sanguis* and many others. They are important pathogens of native valve endocarditis, a very rare infection of heart valves.

Enterococci (*Enterococcus faecalis*, *Enterococcus faecium*)

Enterococcus species is a normal part of the gut flora. *Enterococcus* species can cause urinary tract infections (often associated with urinary catheters), but also endocarditis and wound infections.

The controlled use of vancomycin (VRE) leads to the emergence of vancomycin-resistant enterococci (VRE). Vancomycin-resistant enterococci can cause HAI.

8.3 Gram-Negative Cocci

Neisseria species is the most important example of Gram-negative cocci. There are two predominant pathogens within this genus: *Neisseria meningitidis* and *Neisseria gonorrhoeae*.

*Neisseria meningitidis (***Commonly Called Meningococci***)*
N. meningitidis is the causative agent of epidemic meningitis. This serious infection affects infants and young people in particular. Typical signs include high fever, lethargy/coma and a typical bright rash on the trunk and extremities. This is a very dangerous disease. The patient has to be sent to the hospital. Health authorities have to be notified immediately to install preventive measures. Prophylaxis has to be given to close contact persons.

There are serotypes A, B, C, Y and W135. Serotype B is the most frequent serotype in Central Europe, while serotype C is common in Great Britain or Greece. There are more than 20 different serotypes. 5–10% of the population are asymptomatic carriers with colonization of the nasopharynx; in the cold season, the number may be up to 30%. Transmission is by droplet or contact.

Meningococcal meningitis
After an incubation period of 2–5 days after contact, the infection begins with sudden malaise, high fever, headaches and neck stiffness. The severe course progresses to sepsis and septic shock due to endotoxinemia (spread of bacterial endotoxins in the human body) and disseminated intravascular coagulopathy. Clinical signs on the skin are petechial haemorrhages and also the typical meningococcal rash with map-shaped necrosis in the skin and all organs (purpura fulminans). The most severe form is called the Waterhouse-Friderichsen syndrome (fulminant meningococcal sepsis) with the loss of function of the adrenal glands. The mortality of the Waterhouse-Friderichsen syndrome is up to 95%. If the meningitis remains untreated, the mortality is up to 70%.

A rapid diagnosis is possible by examining a Gram-stained sample of cerebrospinal fluid in the microscope. There are also rapid antigen tests and rapid molecular methods. For correct identification and susceptibility testing, cultures of cerebrospinal fluid and blood have to be done. Close contacts (family, mother, father, siblings and playmates) have to get a chemoprophylaxis.

Vaccination is highly recommended. There are vaccines active against the serotypes A, B, C, Y and W.

Neisseria gonorrhoeae
Neisseria gonorrhoeae is the causative agent of the most common sexually transmitted bacterial infection, gonorrhoea. The reservoir is an infected, often asymptomatic person. Transmission occurs during sexual intercourse. New-borns are infected during birth by their mother.

Gonorrhoea
Approximately 50% of women have symptomatic infection in contrast to 80–90% of men who have ailments like burning painful urination and slimy, creamy discharge (urethritis, prostatitis). Nevertheless, in women gonorrhoea can lead to infection of the annexes and to peritonitis (pelvic inflammatory disease). The inflammation leads to formation of scars and adhesions. Sterility is the consequence. In pregnant women, there is risk of infection of the unborn and amniotic sac (chorioamnionitis), and there is the increased risk of miscarriage. Apart from the urogenital tract and conjunctiva, the pharynx and rectum can be infected too. Besides the infection, *N. gonorrhoeae* elicits immune response in certain individuals, called Reiter's triad (arthritis, conjunctivitis, urethritis).

To prevent eye infection and scarring, new-borns used to be given the so-called Credé's eye prophylaxis. Applying silver nitrate into the eyes of new-borns was carried out since 1881 by the German obstetrician Carl Sigmund Franz Credé (1819–1892). Thus the incidence of purulent conjunctivitis potentially leading to blindness was greatly reduced. Originally Credé's eye prophylaxis was performed with 1% silver nitrate solution. Later on, topical erythromycin or 2.5% povidone-iodine solution was applied within the first hour after birth in the conjunctival sacs of the new-born.

Diagnosis of gonorrhoea is made by examining the Gram-stained smear of the cervix or the urethra in the microscope. Gram-negative diplococci (cocci lying in pairs) are seen. There are also rapid molecular tests.

8.4 Corynebacteria

Corynebacteria are common non-pathogenic commensals being part of the normal microflora on the human skin. However, there is a classic pathogen: *Corynebacterium diphtheriae*, causing diphtheria, a systemic infection involving the upper respiratory tract, particularly the larynx, but also the heart and the nervous system.

Diphtheria
Infected with a phage (virus that infects bacteria), *Corynebacterium diphtheriae* forms a composite toxin (Toxin A and Toxin B). Toxin B has the toxic activity and binds to receptors in the body. The clinical signs and symptoms of diphtheria begin 3–5 days after infection. *C. diphtheriae* is transmitted by droplets in the air or by contact. There is a typical sweet halitosis. In the pharynx there are so-called diphtheritic pseudomembranes that obstruct the airway in young children and lead to breathing difficulties. The patient has massive malaise, fever and swelling of the cervical lymph nodes. The diphtheria toxin is a systemic "intoxication" and causes myocarditis, polyneuritis and even paralysis. The treatment of choice is an antitoxin (horse serum) plus antibiotic therapy with penicillin or macrolide.

Due to the severity of the disease, vaccination with an inactivated vaccine is highly recommended. Diphtheria does not exist in many developed countries, including Austria, due to the vaccination. Cases recorded in developed countries tend to be imported and travel associated. The vaccination is recommended especially when travelling in countries with war, poverty and a poor health system.

8.5 *Bacillus* Species

Bacillus species are anaerobic bacteria. *Bacillus* species are Gram-positive bacteria which grow under anaerobic (without oxygen) conditions and form spores under aerobic (normal air) conditions. Spores are insensitive to heat (up to cooking temperature), to radiation and to disinfectants. There are some sporicidal disinfectants (see Chap. 5 "Disinfection").

The following *Bacillus* species are of clinical importance:

- *Bacillus subtilis*: *B. subtilis* spores are used to test for the activity of a disinfectant against spores in the microbiology laboratory.
- *Bacillus cereus* causes food poisoning, diarrhoea and vomiting. *B. cereus* grows in old food and forms a toxin.
- *Bacillus anthracis* is the causative agent of anthrax. Anthrax is a serious local and systemic infection that can affect the skin, the intestine and the lungs. The lung infection with a septic course form is the most severe form with high mortality. Spores of *Bacillus anthracis* can survive for decades. Anthrax is a notifiable disease and agent of bioterrorism!

8.6 *Clostridium* Species

Clostridium sp. are also anaerobic bacteria and form spores when exposed to ambient air. The most important pathogens of the group *Clostridium* sp. are *Clostridium perfringens*, *C. tetani*, *C. botulinum* and *C. difficile*.

- *Clostridium perfringens* causes gas gangrene (skin, intestine).
- *Clostridium tetani* causes the lockjaw (tetanus). Tetanus occurs not only in adults but also in new-borns. Spores in the dust penetrate into the wounds and form toxin in the tissue under anaerobic conditions. The symptoms are paralysis and muscle spasms (increase of the muscle tone without impairment of consciousness). Typical signs of tetanus are the so-called risus sardonicus and trismus (lockjaw). Neonatal tetanus is very rare and is only found in developing countries. The portal of entry is the umbilical cord wound (it is therefore called the disease of 8th day). Neonatal tetanus has a high fatality rate. Treatment is the application of human hyperimmune serum, muscle relaxants and surgery (debridement). Prophylaxis is vaccination of the mother (transfer of antibodies through breast milk). Vaccination can be applied also during pregnancy. Due to the severity of the disease, the vaccination is highly recommended.

- *Clostridium botulinum* can anaerobically in contaminated food (canned) grow and cause botulism. Botulism is a food poisoning (*botulus* = sausage). *C. botulinum* produces neurotoxin A under anaerobic conditions, e.g. in cans, sausages and other meat products. Neurotoxin A is a potent toxin that leads to paralysis (death by respiratory paralysis) in the amount of 0.1 μg. Symptoms are initially diarrhoea and blurred vision. In wound botulism spores penetrate into the wound and produce toxins that enter the blood stream and cause botulism. Infant botulism: bacterial spores are ingested with food, and after an incubation period of 18–36 h, typical symptoms of dysphagia and diarrhoea start. The therapy used was polyvalent antitoxin.
- *Clostridium difficile* may cause severe diarrhoea in susceptible hosts (see Chap. 14, Infections). *Clostridium difficile* is ubiquitous (anywhere) in the environment and also in small numbers in the intestines of healthy humans. Approximately 50% of children at an age <1 year are colonized with *Clostridium difficile*. *Clostridium difficile* in asymptomatic children should not be treated. Risk factors to develop *Clostridium difficile* infection are age >65 years, renal failures, chemotherapy and antibiotic therapy. The spectrum of *Clostridium difficile* infection ranges from uncomplicated diarrhoea to severe pseudomembranous colitis, perforation of the intestine and death. Infection control measures include the isolation of symptomatic patients, adequate cleaning and disinfection of the environment with a sporicidal disinfectant. Frequent handwashing is required from staff members, visitors and the patients themselves.

8.7 Gram-Negative Rods

Escherichia coli, *Klebsiella* spp. (*K. pneumoniae*, *K. oxytoca*), *Proteus mirabilis*, *Enterobacter* spp. (*E. cloacae*, *E. aerogenes*), *Citrobacter* spp. (*C. freundii*), *Serratia* spp. (*S. marcescens*) and many others are summarized under the term "enterobacteria". They are the most common cause of bacterial infections in humans. The most common infections caused by enterobacteria are urinary tract infections, sepsis, wound infections and even pneumonia in patients with mechanical ventilation.

Enterobacteria are normally part of the gut flora. They are just a very small fraction of it compared to anaerobes. The most common representatives of enterobacteria are listed below:

There is a group of bacteria called "non-fermenters". These non-fermenting bacteria cleave only a few sugars and amino acids in contrast to enterobacteria. The most common pathogens among the non-fermenters are *Pseudomonas aeruginosa*, *Acinetobacter baumannii* complex and *Stenotrophomonas maltophilia*.

8.7.1 *Escherichia coli*

E. coli was the first bacterium to be detected. The laboratory strain K8 is still one of the working horses for laboratories. *E. coli* grows very well under aerobic conditions. *E. coli* can multiply very quickly. One bacterial *E. coli* cell multiplies so often during 12 h that after 12 h there are 343 597 383 680 *E.coli* cells.

Escherichia coli is part of the normal physiological intestinal flora. *E. coli* is a faecal contamination in drinking water. *E. coli* is the most frequent cause of healthcare-associated infections (44%). *E. coli* is the common pathogen of urinary tract infections and sepsis. Other infections are peritonitis, cholangitis and sepsis and meningitis in new-borns.

Some strains of *E. coli* may cause diarrhoea. They are therefore called diarrhoeagenic *E. coli*:

- Enterohaemorrhagic *E. coli* (EHEC) O157, O104 causes the haemolytic uremic syndrome.
- Enteropathogenic *E. coli* (EPEC) causes diarrhoea in infants.
- Enteroinvasive *E. coli* (EIEC) causes non-*Shigella* dysentery.
- Enterotoxigenic *E. coli* (ETEC) causes traveller's diarrhoea.

Improper storage, improper production and use of food (beef, raw milk) can lead to food poisoning by *E. coli*. The therapies of choice are broad-spectrum penicillins, cephalosporins of the second or third generation or quinolones. However, resistance to quinolones may be as high as 20% of the isolates or up to 30% to third-generation cephalosporins. These enterobacteria produce extended-spectrum beta-lactamases (ESBL) that can be up to 30%.

8.7.2 *Klebsiella* Species (*Klebsiella pneumoniae*, *Klebsiella oxytoca*)

Klebsiella sp. can also cause urinary tract infections, sepsis, cholecystitis, as well as pneumonia, lung abscess and otitis media. *Klebsiella pneumoniae* has higher resistance to third-generation cephalosporins and quinolones.

Klebsiella granulomatis is the causative agent of a sexually transmitted disease (STD) called granuloma inguinal or donovanosis. Normally localized in the intestinal tract, *Klebsiella granulomatis* is unremarkable. However, if it is translocated to genital tract, there are painless ulcers with tissue destruction and severe bleeding without swollen glands in the groin. The treatment of choice consists of tetracycline or macrolides.

8.7.3 *Proteus* Species

Proteus species include *P. mirabilis*, *P. vulgaris* and *P. penneri*. *Proteus* species is highly mobile and forms typical appearance on culture plates with a very unique smell. *Proteus* species are found in the gut and in the groundwater. Like *E. coli*, *Proteus* species is often the pathogen of urinary tract infections, sepsis or wound infection.

8.7.4 *Salmonella* Species

Salmonella species are pathogens causing diarrhoea in most cases. Only *Salmonella typhi* and *Salmonella paratyphi* cause a sepsis-like illness, typhoid fever.

There is a number of *Salmonella* species. The majority of *Salmonella* sp. cause diarrhoea, primarily *Salmonella enteritidis* and many others. Generally, this diarrhoea is self-limiting. Treatment is oral rehydration. *Salmonella typhi* and *Salmonella paratyphi* are the causative agents of typhoid and paratyphoid fever. Usually, typhoid or paratyphoid fever is acquired in tropical regions. The diagnosis is made by blood culture or by serological testing. Treatment of choice depends on the susceptibility pattern because there are resistant strains.

Salmonella diarrhoea is a zoonosis. *Salmonella* sp. can be found on poultry meat and eggs. There can be asymptomatic intestinal colonization in humans. Salmonellosis has to be reported to the health authorities. Serving, handling or cooking of food is prohibited for patients with *Salmonella* disease or colonization.

8.7.5 *Shigella* Species

Shigella dysenteriae is the causative agent of bacterial dysentery. Bacterial dysentery is a serious disease of the colon (colitis) with muco-bloody diarrhoea, painful bowel motions and occasional vomiting. *S. sonnei*, *S. flexneri* and *S. boydii* cause diarrhoea, but rarely a dysenteric colitis. The source of infection is contaminated food and sometimes via contact with contaminated items. Flies may be vectors. Actions like handling, cooking or serving of food are prohibited for patients with *Shigella* infection.

8.7.6 *Yersinia* Species

Yersinia are Gram-negative rods. There are three medically relevant *Yersinia* species.

In humans *Yersinia pestis* causes the plague, a serious sepsis with involvement of the lymph nodes (bubonic plague) and sometimes of the lungs. But plague is a zoonosis because *Y. pestis* is found in asymptomatic gerbils. These gerbils are restricted to some parts of the world, e.g. Mongolia or Southeastern United States. If rat fleas (*Xenopsylla cheopis*) feed on gerbils and on rats, *Y. pestis* is transmitted to rats. However, *Y. pestis* causes disease in rats and fleas. Fleas start to feed on other mammals and thus spreading plague even to humans.

In the Middle Ages, plague was a feared epidemic. However, plague can be treated with antibiotics. Nowadays, there may be individual cases of plague in endemic areas if there is a transmission when handling wild symptomless gerbils carrying the *Y. pestis*.

Y. enterocolitica causes enteritis with colicky pains and loose stools. Usually it is isolated in children with severe diarrhoea. Infection with *Y. enterocolitica* can lead to sepsis and abscess formation, but also attack bones or the heart.

Y. pseudotuberculosis causes enlarged mesenteric lymph nodes and pain in the right lower quadrant of the abdomen. Because of the localization, it is mimicking appendicitis; thus it is also called pseudoappendicitis.

8.7.7 *Vibrio* Species

Vibrio are Gram-negative rods that can commonly occur in tepid, salty water.

Vibrio cholerae is endemic in tropical areas. Cholera outbreaks occur primarily in areas with poor hygienic conditions (lack of sanitation, no fresh water), environmental emergencies or major population movements (refugees). *V. cholerae* is the causative agent of Asiatic cholera. Cholera is a toxic diarrhoea with up to 20 l of fluid loss per day. Left untreated, the mortality is up to 50% particularly in children. *V. cholerae* produces a heat-stable cholera toxin which stops the water reabsorption in the colon. The transmission of *V. cholerae* occurs through contaminated water. Outside of water the pathogen dies rapidly. There are virtually no healthy carriers. Infection control and prevention measures include strict water hygiene (boil water in countries with unsafe water) and adequate sewage. The treatment of cholera is rehydration. Antibiotic therapy kills the pathogen but has only small effect on the diarrhoea because diarrhoea is due to the toxin. For practice, it is important to take the travel history of expectant mothers with watery diarrhoea. *V. cholerae* is a notifiable disease to healthcare authorities.

8.7.8 *Haemophilus* Species

Haemophilus are Gram-negative rods that are part of the normal flora of the respiratory tract. *Haemophilus influenzae* is a pathogen of upper and lower respiratory infections but also of bacterial meningitis. In small children between 6 months and 4 years, *H. influenzae* can cause acute tonsillitis (angina), sinusitis, otitis media and pneumonia but also a bacterial purulent meningitis and endocarditis. The treatment of choice consists of aminopenicillin plus beta-lactamase inhibitor or cephalosporins. There is an efficacious vaccine.

Haemophilus ducreyi is the causative agent of the sexually transmitted disease, chancroid. This sexually transmitted disease is mainly found in tropical regions. The patients with chancroid present with roundish greasy-occupied ulcers on the genitals and swelling of the local lymph nodes. The treatments of choice are quinolones, macrolides and third-generation cephalosporins.

8.7.9 *Bordetella pertussis*

Bordetella pertussis is the causative agent of whooping cough (pertussis). Whooping cough is common in children. But also adults may acquire and spread pertussis. The bacteria are easily spread in droplets through the spasmodic cough attacks. In infants these coughing fits can lead to apnoea. Therefore, mortality can be up to 2% in first year of life. There is an effective vaccine against *Bordetella pertussis* (acellular vaccine) for children and adults.

8.7.10 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a non-fermenting Gram-negative rod and commonly found in the humid environment but also in the gut of humans and animals. *P. aeruginosa* is an opportunistic pathogen and causes severe sepsis in patients with leukaemia. *P. aeruginosa* is an important pathogen of healthcare-associated infections and in infections associated with implanted material because it easily forms biofilms on material surfaces. The most common infections caused by *P. aeruginosa* are urinary tract infections, wound infections, infections of the outer ear and infections of large burn wounds. Children with cystic fibrosis, an inborn genetic disease with failure to clear the mucus from the respiratory tract, become colonized and infected with *P. aeruginosa*.

In the microbiology laboratory, *P. aeruginosa* is identified at the first glance to the agar plate because it has an impressive colour that ranges from lime green to petrol blue and his sweet pungent odour. *P. aeruginosa* produces the enzyme oxidase. Test strips with an oxidase substrate that are commonly used in microbiology become a blue colour when pressed on the colonies of *P. aeruginosa*.

A prominent feature is that *P. aeruginosa* is not susceptible to many antibiotics. Exposed to antibiotics *P. aeruginosa* rapidly develops resistance.

Acinetobacter baumannii complex and *Stenotrophomonas maltophilia* are non-fermenters, but they are oxidase-negative. The colonies on the agar plate are unspectacularly greyish. However, both are resistant to most antibiotics commonly used for treatment. They can be selected by the extensive use of antibiotics and cause serious infections, sepsis, pneumonia and urinary tract infections, in immunocompromised and intensive care patients.

Acinetobacter baumannii complex survives well in the environment and may be easily transmitted and cause outbreaks in the hospital setting. Thus, infection prevention and control measures including hand hygiene and environment cleaning and disinfections have to be observed meticulously.

8.8 Other Rod-Shaped Bacteria

There are bacteria which are not characterized by Gram staining because their cell wall has different compositions, e.g. mycobacteria, or they are living within the human cells, e.g. chlamydia.

The most important pathogens are mycobacteria, *Treponema pallidum*, *Rickettsia* and *Borrelia* species.

8.8.1 Mycobacteria

Mycobacteria are acid-fast bacilli because they keep the red stain in the Ziehl-Neelsen staining. Their lipid-rich outer cell membrane makes mycobacteria resistant to environmental noxes. The treatment of mycobacterial diseases differs significantly from the treatment of other bacterial infections. Therefore, a distinction between bacteria and mycobacteria is pivotal.

There are many mycobacterial species. A distinction between "fast-growing" and "slow-growing" mycobacteria gives the first clue to identification. "Rapidly growing" mycobacteria grow within 14 days on the special growth media. "Slow-growing" mycobacteria require up to 6 weeks to grow. Therefore, for the diagnosis of mycobacterial diseases, molecular PCR-based diagnostic tests are particularly important.

Human pathogens of the genus *Mycobacteria* are *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium africanum*, *Mycobacterium microti*, *Mycobacterium leprae* and *Mycobacterium ulcerans* (Buruli ulcer). Among the so-called atypical mycobacteria, the most important is *Mycobacterium avium intracellulare* complex.

Mycobacterium tuberculosis
Mycobacterium tuberculosis is one of the most important pathogens worldwide. It is the causative agent of tuberculosis, a world-occurring disease. For 90% of healthy people, infection with *M. tuberculosis* is asymptomatic. However, there is no complete elimination of the bacteria. Mycobacteria can survive intracellularly in macrophages for life. When the immune system is weakened, e.g. in AIDS, alcoholism, iatrogenic immunosuppression or very old age, there may a reactivation of the endogenous infection.

Tuberculosis
Tuberculosis is common in countries with low income and a high rate of AIDS. About 150 years ago, tuberculosis was very common in Europe, especially in urban areas due to famine and poor housing conditions. By improving living conditions, and with other preventive measures, the tuberculosis epidemic in Europe was contained in the early twentieth century. Currently, there are up to 1.7 billion people infected. Not all infected people have a visible disease but a latent infection that can erupt

easily when the patient becomes immunocompromised. Estimates show that 20 million people have a so-called open tuberculosis of the lungs. Open pulmonary tuberculosis is associated with a high risk to spread *M. tuberculosis* by coughing. Transmission of mycobacteria is airborne meaning that the infectious particles remain longer in the air. Thus open pulmonary tuberculosis is of hygienic relevance. Patients with open pulmonary tuberculosis have to be isolated in a special isolation room that should only entered with special tightly fitting masks (class 2, FFP-2, N90). Contact persons have to be identified and followed up. Three million persons die each year from the effects of tuberculosis.

Classification of Tuberculosis

- Primary tuberculosis: Infection occurs via air directly through droplets. Primary infection affects usually the lungs. There is a small initial lesion and lymph node.
- Secondary tuberculosis: When reactivated there is an endogenous spread of pathogens in immunocompromised host in almost all organs. Symptoms are nonspecific.

Signs and symptoms include subfebrile temperatures (below 38.3°), night sweats, weight loss, swollen lymph nodes, fatigue and weakness, cough or dry cough with little expectoration.

In immunocompromised persons, a severe disease may occur in varying manifestations including sepsis, tuberculous meningitis or organ involvement which looks like a tumour in the computed tomography.

Diagnosis of Tuberculosis

Microbiological diagnosis includes direct detection and visualization of acid-fast bacilli by staining of the material (sputum, aspirate, etc.) using the Ziehl-Neelsen stain. The test is easy but less sensitive compared to the PCR test. Cultures will be positive after up to 6 weeks. Serology testing is done using specific lymphocyte reaction to a *Mycobacterium tuberculosis* antigen (IGRA, interferon-gamma reaction assay). This test replaces the tuberculin from the screening of people at risk.

Previously a tuberculin test was performed (Mantoux text). This test is based on the cross-reaction of an infected with tuberculosis people to the intracutaneous administration of *Mycobacterium bovis*—antigens. The test is nonspecific and only says that the individual had contact with *Mycobacterium tuberculosis* or *Mycobacterium bovis* (BCG). The test does not indicate whether an acute clinical infection or years past healed, clinically silent, infection. A negative test indicates that a patient has never had contact with tuberculosis. However, in severely immunocompromised patients, the reaction is also negative despite active clinical infection.

Tuberculosis has to be reported to health authorities under the Tuberculosis Act. Patients with tuberculosis are regularly monitored by the health authorities.

Treatment of Tuberculosis
The treatment of tuberculosis consists of a combination of 3–4 antituberculous over 3–6 months. Initially a combination of isoniazid, rifampicin, pyrazinamide and ethambutol is administered for 2 months, followed by a combination of isoniazid and rifampicin for the next 4–7 months. In South and East Asia, Eastern Europe and some African countries, there are *Mycobacterium tuberculosis* strains that are resistant to this standard therapy, multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB). For practice it is important to know the travel history or the country of origin of the expectant mothers to identify a possible risk for these infections and to refer them to a dedicated tuberculosis centre for further diagnostics and treatment if indicated.

8.8.2 *Treponema pallidum*

Treponema pallidum is a spirochaete bacterium. Spirochaetes have a helical form and look like a screw. *T. pallidum* is the causative agent of syphilis, one of the most important sexually transmitted infections. The transmission from an infected person is through sexual intercourse. The incubation period of syphilis is about 21 days (10–90 days). *Treponema* enters the bloodstream and is distributed in the whole body and all organs and the brain. Congenital syphilis develops when infection occurs during the later stages of pregnancy. *Treponema* is transmitted to the foetus through the placenta, infecting the foetus, and the child is born with congenital syphilis.

The diagnosis is made with dark-field microscopy looking on material from syphilitic lesion on a glass slide. *T. pallidum* can be seen as corkscrews moving rapidly around. However, be careful to use always gloves for examination and sampling because *Treponema* are very infective and may enter through very tiny lesions of the skin or the mucosa.

More commonly the diagnosis is made using serological tests. There is a screening test, the VDRL test, the TPHA (*Treponema pallidum* haemagglutination test) and the treponemal antibody tests (FTA-ABS) that can detect both IgG and IgM antibodies. FTA-ABS-IgM antibodies are present in a very recent infection.

Syphilis
There are four stages of syphilis.

- Stage I:
  About 3 weeks after infection, a painless firm papule develops and evolves to an ulcer with firm ground and sharp borders at the site of the primary infection as well as a painless swelling of the local lymph node. The ulcer ground is infested heavily with *Treponema* bacteria and very infectious. The painless ulcer is also called hard chancre, and ulcer plus the lymph node is called primary complex of syphilis.

- Stage II:
  About 4–10 weeks after infection, there is generalization of the diseases with fever, malaise, weakness, weight loss, hair loss and headache. There are typical red flat to papulous lesions on the skin of the trunk, extremities and particularly on palms and soles. Further there are wart-like lesions, so-called condylomata lata, on the mucosae of the mouth and at the genitalia. These lesions are full with bacteria and thus very infectious. The symptoms resolve after 6–10 weeks even without therapy.
- Latent syphilis:
  After stage II syphilis may become latent, i.e. symptomless. The latency period can last up to years. Latent syphilis is defined as a positive serologic test but no symptoms of disease.
- Stage III:
  The final stage of syphilis is also called lues or gummatous syphilis. In this stage gummas develop. Gummas are painless tumours consisting of granulomatous immune reaction to the *Treponema*. The gummas can be very large. The symptoms are dependent on the localization of the gummas because gummas can develop in all organs including the central nervous system. Neurosyphilis manifests as meningitis (involvement of the meninges), meningovascular syphilis, general paresis or tabes dorsalis with various neurological symptoms. The symptoms are neuropathic pain, ataxia, apathy, seizures, general paresis and dementia. About a third of untreated patients develop stage III symptoms. Stage III syphilis is not infectious.
- Congenital syphilis:
  Two-thirds of new-borns infected with syphilis are born without symptoms. Symptoms develop during the first years of life and include enlargement of the liver and spleen, rash, fever, neurosyphilis and pneumonia. If untreated, children may develop the typical saddle nose deformation or sober shins. Infection during pregnancy is also associated with miscarriage.
  Because of these serious sequelae, syphilis test is done during pregnancy in Austria under so-called mother-child pass.

In some developed countries, cases of syphilis have to be reported to health authorities if the patients deny treatment of him-/herself and the partners. Treatment must also include the sexual partners.

8.8.3 *Rickettsiae*

Rickettsia is a genus of nonmotile bacteria that can have the shape of cocci, rods or threads (up to 10 μm long). They are obligate intracellular parasites because *Rickettsia* survive, grow and replicate within the cytoplasm of eukaryotic host cells (typically endothelial cells). They cannot live in the environment. *Rickettsia* cannot be cultured on agar plates but on tissue cell cultures only. Culture is therefore not done in the routine clinical microbiology laboratory.

Diagnosis is made by serology tests only.

Rickettsia species are transmitted by vectors, commonly arthropods, including chigger, ticks, fleas and lice. *Rickettsia* species are the pathogens of "spotted fevers" including rickettsialpox, African tick bite fever, Rocky Mountain spotted fever, Queensland tick typhus (Australian tick typhus) and fever boutonneuse. The patients have fever, rash and most often a typical lesion at the site of tick bite (eschar). *Rickettsia prowazekii* causes epidemic typhus (trench fever), recrudescent typhus and sporadic typhus, and *Rickettsia typhi* (worldwide) causes endemic typhus (murine typhus). A rickettsia-like pathogen *Orientia tsutsugamushi* causes scrub typhus.

In Austria, rickettsial disease is uncommon and only to be suspected in travellers returning from outdoor activities in Africa, Australia, the United States and Asia.

8.8.4 *Borrelia* Species

Borrelia sp. are *Spirochete* bacteria. Like *Rickettsia* they are obligate intracellular parasites. They cannot live in the environment. Culture of *Borrelia* is only done in specialized research laboratory. In the clinical routine, serology test (antibodies against *Borrelia*) is performed.

B. recurrentis (vector: lice) and *B. duttonii* (vector: ticks) are pathogen causing relapsing fever. These diseases occur in humans in bad circumstances before (wars, prisons, refugees).

Borrelia burgdorferi in the United States and *Borrelia afzelii* or *Borrelia garinii* in Europe are pathogens of tick-borne borreliosis. The vectors are ticks. There are two sylvatic cycles linked to life cycle of the tick vectors and wild animals.

Lyme Disease (Borreliosis)

Borreliosis is a zoonosis. First, borreliosis was named Lyme disease after the geographical site of the first diagnosis. At the site of the tick bite, a typical lesion with a reddish border and pale centre erythema chronicum migrans develops about 2–3 weeks later. If spreading of the borrelia into whole body occurs, there may be several manifestations like acrodermatitis atrophicans involving the fingers and the hand as well as myocarditis, cardiomyopathy, arrhythmia, arthritis, arthralgia, meningitis, neuropathies and facial nerve palsy.

Treatments of choice are penicillins and tetracycline.

8.8.5 *Chlamydia* Species

Chlamydia are cell wall-less bacteria. There are several *Chlamydia* or *Chlamydia*-similar species, but the most important *Chlamydia* is *Chlamydia trachomatis*. *Chlamydia* are characterized by a complex life cycle. They live a "parasitic" life in cells. They are obligate intracellular parasites because *Chlamydia* survive, grow and replicate within the cytoplasm of eukaryotic host cells. *Chlamydia* can therefore

survive in the cells. For propagation the chlamydia are released from the cell and infect the next cell.

Chlamydophila psittaci is the causative agent of psittacosis. The reservoirs are birds, so it is a zoonosis (ornithosis, psittacosis). Transmission to humans can lead to pneumonia and rarely a haematogenous spread in the liver, spleen and central nervous system. The transmission is airborne dust, rarely minor injuries in the laboratory. The incubation time is 4 to 14 days. The diagnosis is a serological blood test by detecting antibodies to *Chlamydophila psittaci*. The treatments of choice are macrolides and tetracycline.

Chlamydophila pneumoniae is occasionally exciting an atypical pneumonia in humans. There will be an increase with age colonization of the respiratory tract with *Chlamydophila pneumoniae*, so it is unclear whether this bacterium is causative pathogen. It is estimated that 10% of the atypical pneumonia is caused by *C. pneumoniae*. Transmission is airborne from person to person. 70% of all infections with *C. pneumoniae* are asymptomatic.

Chlamydia trachomatis is a common cause of chlamydia infections associated with different serotypes. There are distinct clinical syndromes:

- Scarring conjunctivitis

  Chlamydia trachomatis serovars A, B, Ba and C cause scarring conjunctivitis that leads to blindness in dry subtropical zones (desert). According to WHO it is estimated that 1.9 million people are affected by blindness or visual impairemetn due to trachoma. Overall about 158 million people currently live in endemic areas for trachoma and are therefore at risk of blindness due to this disease. The underlying causes are poor sanitation and lack of water, and the transfer is made by contaminated items, by flies or by direct contact among people.
- Chlamydial urethritis (unspecific urethritis)

  Chlamydia trachomatis serovars D–K are the most frequent cause of sexual disorder which is known as urethritis or cervicitis in the United States and Western and Northern Europe. The test has been done by means of molecular-biological tests in the smear from the urethra or cervix. The sessions are held with macrolides and tetracycline and will include all partners. New-borns can be infected during birth. Possible long-term consequences are chronic scarring caused by chronic inflammation and consequent infertility. In case of an excessive immune response reactive arthritis can occur. The symptom complex in urethritis, arthritis and conjunctivitis is called Reiter's syndrome. Epidemiologically, it is estimated that there are 3–5 million new infections of STD *Chlamydia trachomatis* D–K in the United States. Two-thirds of women are asymptomatic. In men, it is more common to clinical symptoms with urethral discharge. In the women occur subacute infections with nonspecific abdominal pain, sometimes urethritis, but also endometritis, salpingitis. Scarring can cause ectopic pregnancies and infertility. The incidence of arthritis is 1–3%.
- Lymphogranuloma venereum

  Chlamydia trachomatis serovars L1–L3 are the causative agent of the venereal disease lymphogranuloma venereum. It creates small ulcerative primary lesion ("herpetiform"). Finally, there is a painful swelling and purulent melting of the regional lymph nodes.

Suggested Readings

Bennett JE, Dolin R, Blaser MJ. Mandell, Douglas and Benett's principles and practice of infectious diseases: expert consult premium edition—enhanced online features and print. 8th ed. Oxford: Elsevier; 2014. ISBN-10: 1455748013. ISBN-13: 978-1455748013.

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Farrar J, Hotez P, Kang G, Lalloo D, White NJ. Manson's tropical diseases. 23rd ed. Oxford: Elsevier; 2013. ISBN-10: 0702051012. ISBN-13: 978-0702051012.

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