Fundamentals of Microbiology and the Science of Infection

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1 Fundamentals of microbiology

1.1 Microorganisms (smallest living creatures)

What are microorganisms?

Microorganisms are tiny living creatures that cannot be seen with the naked eye; they are visible only under the microscope (from around 1000-fold magnification). These tiny creatures include:

- Bacteria
- Viruses
- Fungi
- Protozoa

A common feature of all microorganisms (= microbes, germs) is that they cannot be seen, felt or tasted. And that is just what makes things so difficult. Objects, e.g. our hands, which appear clean to our eyes, can harbour several microorganisms.

Humans classify microorganisms in terms of their being beneficial or harmful (meaning, those that are beneficial or harmful to man). There are many different species of bacteria, viruses, fungi and protozoa, most of which are not at all known to us, and the majority are neither beneficial nor harmful to us humans.

Only a small proportion of microorganisms cause disease (= are pathogenic), and most do not cause disease (= are apathogenic).

- Microorganisms are tiny living creatures that can be seen only under the microscope. They include bacteria, viruses, fungi and protozoa. Some can cause disease, while others are beneficial to us humans.
1.1.1 Where microorganisms are to be found

Microorganisms are to be found everywhere in nature. Even under seemingly inclement conditions (extreme cold, heat or drought) some microorganisms can survive and even multiply.

There is virtually nowhere on earth where there are no bacteria; microorganisms have been found even in geysers (hot springs, e.g. in Iceland) as well as in the eternal ice of polar regions.

In the ice of an Antarctic sea, US researchers found bacteria and algae that were almost 3000 years old. "When we warmed them up a little, they came back to life", announced Peter Doran, the head of the research expedition at the University of Illinois.

- Microorganisms are to be found e.g. in the soil, in and on living creatures, in the water, in the air, etc.

1.1.2 Bacteria

- Bacteria are microscopically small

They measure around one-thousandth of a millimetre in size, i.e. one would have to use 1000 bacteria, such as threading a pearl necklace, to get one millimetre.

The microscope must use thousand-fold magnification, so that one can see a bacterium measuring one millimetre.
- **Bacteria are made of a single cell**

![Diagram of bacterial structure]

- **There are many different species of bacteria**

![Diagram of different bacterial shapes]

Bacteria can be distinguished on the basis of their shape, with a distinction being made between spherical bacteria (cocci), rod-shaped bacteria and spiral bacteria.

- **Bacteria must take in food, just like every other living creature.**
• **Bacilli are one species of bacteria**
In the colloquial (informal) language of some countries, the term “bacilli” is often used instead of “bacteria”. Bacilli (singular: bacillus) are one species of bacteria and, in fact, are the first genus to have been identified as pathogens (that disease was anthrax, discovered by Robert Koch). Hence, all bacilli are bacteria but not all species of bacteria are bacilli. The situation is similar to that of Kleenex, where “Kleenex” tends to be used for all disposable paper handkerchiefs. But while every Kleenex is a paper handkerchief, not every paper handkerchief is Kleenex.

- Bacteria are microscopically small and are composed of a single cell
- Bacteria must take in food just like every other living creature
- There are many different bacteria
- Bacilli are one genus of bacteria

• **Some bacteria are masters of survival and can change into spores.**
We are familiar with the term “spores” from the fungi. Fungal spores are, so to speak, the seeds of the fungus, whereas bacterial spores are something very different and have absolutely nothing in common with fungal spores.

Some bacteria can change into spores. Under unfavourable living conditions, the bacterium rolls up into a capsule and thus survives the inclement conditions. These masters of survival are called spore-forming bacteria.

Under conditions when a normal bacterial cell would die, the spore can generally survive and remain viable for a very long time (in extreme cases even for centuries). If living conditions become more favourable, the spore can revert to being a bacterial cell.

Bacterial spores could be compared to a hibernating hedgehog. When the weather becomes too cold in autumn for the hedgehog and it is unable to find any food, it seeks out a bed of leaves where it survives the cold period. The tortoise withdrawing into its shell is another such example.
Normal bacterial cells are heat sensitive, with most dying at temperatures above 60 °C. Bacterial spores can only be killed by very **high temperatures, e.g. in a sterilizer**. Examples of spore-forming bacteria are the causative organisms of anthrax, gas gangrene and tetanus.

- Some bacteria are masters of survival and can change into spores
- Bacterial spores are something very different from fungal spores
- Bacterial spores can only be killed by very high temperatures (sterilizer)
- Bacteria that are able to form spores are called spore-forming bacteria (e.g. the causative organisms of anthrax, gas gangrene and tetanus)

- **Some bacteria can produce toxins.**
  Another special characteristic of certain species of bacteria is their ability to produce **toxins**. These toxins are substances released by bacteria and can be harmful to humans.
  Example: the most potent toxin in nature is the one produced by a bacterium called Clostridium botulinum. Only 2 milligrams (1/1000 of a gram) of this botulinum toxin (better known as Botox) would be enough to kill up to half a million people.
  Food poisoning is caused by the toxins of certain bacteria e.g. *Staphylococcus aureus*.

- **Bacteria can be found just about anywhere**
  Bacteria can be found in almost every environment (the air, soil, water). But certain living conditions are particularly suitable for these living creatures and promote their rapid multiplication.

**Favourable living conditions for bacteria are as follows:**

- **Humidity**
- **Warmth**
- **Good supply of nutrients**

  e.g., moist cleaning cloths, moist locations (water taps), unrefrigerated foodstuffs
Unfavourable living conditions for bacteria are as follows:

- **Dryness**
- **Heat**
- **Cold**
- **No food supply**

E.g. refrigerator, deep freezer, boiling, pasteurization

Bacterial spores can only be killed by very high temperatures (e.g. steam sterilizer 134 °C / 3min)

Normal bacterial cells are heat sensitive, with most dying at temperatures above 60 °C

Cold cannot affect bacteria but, depending on the temperature, they will not, or only slowly, multiply

One could say that bacteria “sleep” in the deep freezer
Microorganisms Worksheet

Why does food spoil (“go off”), why do e.g. leaves rot?

Why does food not spoil in the deep freezer?

Why does food keep longer in the refrigerator?

Why does food spoil quickly in the sun?

Why do conserves keep almost forever?

Why does raw rice keep almost for ever?
Please state where you think bacteria are to be found and try to estimate their number, and whether harmful or beneficial to humans.

Note: what is meant is the original state (e.g. bread rolls straight from the oven are sterile) and normal status (e.g. a healthy person). Multiple answers are possible.

<table>
<thead>
<tr>
<th>Bacteria present in normal state</th>
<th>No</th>
<th>Yes</th>
<th>Many</th>
<th>Rather few</th>
<th>Harmful</th>
<th>Beneficial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hands</td>
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<tr>
<td>Urine</td>
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<tr>
<td>Wound with pus</td>
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<tr>
<td>Stools</td>
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<tr>
<td>Sputum (saliva)</td>
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<tr>
<td>Skin</td>
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<tr>
<td>Blood</td>
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<td>Tap water</td>
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<td>Yoghurt</td>
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<tr>
<td>Raw milk</td>
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<tr>
<td>Beef soup</td>
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<td>Boiled noodles</td>
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<tr>
<td>Raw chicken</td>
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<tr>
<td>Eggs</td>
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<tr>
<td>Conserves</td>
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<tr>
<td>Bread rolls</td>
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<tr>
<td>River water</td>
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<tr>
<td>Soil</td>
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<tr>
<td>Floor</td>
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<tr>
<td>Cleaned surface</td>
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<tr>
<td>Washbasin</td>
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<tr>
<td>Surgical instrument</td>
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<tr>
<td>Doorknob</td>
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<tr>
<td>Coins</td>
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<tr>
<td>Air</td>
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</tr>
</tbody>
</table>
Worksheet: contact culture

- Bacteria can be cultured in the laboratory

In the microbiology laboratory bacteria are cultured and precisely identified so that the correct medication can be selected.

The presence of bacteria is now to be investigated

The contact culture contains nutrient medium (nutrients for the bacteria)

Once the contact culture has been taken, it is incubated at 37 °C for 48 hours in an incubator.

<table>
<thead>
<tr>
<th>Suggestions for sites to be swabbed for culture:</th>
<th>Results, Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hand before disinfection</td>
<td></td>
</tr>
<tr>
<td>Hand after disinfection</td>
<td></td>
</tr>
<tr>
<td>Forehead</td>
<td></td>
</tr>
<tr>
<td>Floor</td>
<td></td>
</tr>
<tr>
<td>Toilet seat</td>
<td></td>
</tr>
<tr>
<td>Bank note</td>
<td></td>
</tr>
<tr>
<td>Window pane</td>
<td></td>
</tr>
</tbody>
</table>

- Several bacteria live in humans

Bacteria also live on and in our body, without making us sick.

For example, more than 100 different species of bacteria are to be found in our intestines. Each gram of stools contains up to 10 billion bacteria (e.g. *Escherichia coli*, *Klebsiella*).
Some bacteria can cause disease

There are many different species of bacteria, but only very few can cause disease in humans.

Examples of diseases caused by bacteria: salmonellosis, typhoid fever, urinary tract infections (bladder infection), wound suppuration (wound with pus), scarlet fever, tuberculosis, etc.

- Bacteria are to be found just about anywhere
- Favourable growth conditions: warmth, humidity, good supply of nutrients
- Unfavourable growth conditions: cold, heat, dryness, no nutrients
- Bacterial spores can only be killed by very high temperatures (sterilizer)
- Normal bacterial cells are heat sensitive, with most dying at temperatures above 60 °C
- Many bacteria also live on and in humans
- Certain bacteria can cause disease
1.1.2.1 Multiplication of bacteria

Bacteria multiply by dividing. Two daughter cells are formed from one mother cell, so there are no Mum and Dad bacteria. The time taken to divide will depend on the species of bacterium and on the environmental conditions. Example: under favourable conditions, one cell of the intestinal bacterium *Escherichia coli* divides around once every 20 – 30 min. At that multiplication rate one bacterial cell can give rise to several million other cells within the space of a few hours.

With 2 divisions per hour:

<table>
<thead>
<tr>
<th>Time</th>
<th>Number of Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline (starting point)</td>
<td>1 cell</td>
</tr>
<tr>
<td>1 hour</td>
<td>4 cells</td>
</tr>
<tr>
<td>2 hours</td>
<td>16 cells</td>
</tr>
<tr>
<td>5 hours</td>
<td>4,096 cells</td>
</tr>
<tr>
<td>10 hours</td>
<td>1,048,576 cells</td>
</tr>
<tr>
<td>17 hours</td>
<td>17,179,869,184 cells</td>
</tr>
</tbody>
</table>

After 17 hours one single cell will have given rise to **17 billion 179 million 869 thousand 184 bacterial cells**

▶ Bacteria multiply enormously fast
**Mathematical games with bacteria**

Calculation examples can help to gain an idea of how quickly bacteria multiply making us gasp in astonishment.
All examples can be mastered with just a basic grasp of mathematics. A calculator will be useful but some calculators do not, unfortunately, have enough decimal places.

1) Let’s assume that a human being could reproduce as quickly as bacteria, after how many hours would a 100-inhabitants’ village have given rise to a city with a population of one million? (Bacteria divide around once every 30 minutes under favourable conditions)

2) Every 30 minutes a new generation of bacteria is formed. How many generations are formed in one week?
3)
In what epoch would our ancestors who were born 336 generations before us live? (let’s assume that it takes 25 years for each new generation.)

At that time:
Great Britain is separated from continental Europe. This is because the glaciers melt and the sea level rises accordingly. A massive earthquake takes place at Storegga in southwest Norway, flooding settlements in Scotland. The Bosporus is flooded, saltwater pours into the Black Sea, causing the sea level to rise by more than 100 meters. Several coastline settlements are flooded. It is thought that this catastrophe serves as a backdrop to the Deluge in the Gilgamesh Epos and in the Bible. Monsoon climate prevails in the Mediterranean region. Irrigation of fields in Mesopotamia, ceramics in Mehrgarh, south Asia, agriculture in the Nile Valley, Egypt, Cultivation of rice in Asia, invention of the wheel, invention of the plough, foundation of temples in south Mesopotamia.

Based on this example it can be demonstrated why bacteria are so adaptable and why e.g. resistance can develop relatively fast. Accordingly, today 70 - 80 % of staphylococci are resistant to penicillin which was first used in 1945 (discovered in 1928 Sir Alexander Fleming).

4)
After 17 hours under favourable conditions one bacterium will have given rise to 17 billion. Let’s assume that one bacterium weighs 1 gram, how many kilos or tons would be formed? Let’s also assume that an automobile weighs one ton and is 4.5 m long, how long would the traffic build-up be?
5) You leave your workplace at 4 pm but, unfortunately, you forget about that wet cloth harbouring 10,000 bacteria. How many bacteria will await you when you start work the next morning at 6 am?

6) After 17 hours under favourable conditions one bacterium will have given rise to 17 billion bacteria. Let’s assume you had 17 billion euros in 1 euro coins and wanted to count these. You count one coin per second, how long would it take you? (Please first estimate, then calculate)
1.1.3 Viruses

Viruses are much smaller than bacteria, they measure from 0,000,002 to 0,000,030 mm = 2-30 nanometres (nm). They cannot be seen even using a normal microscope. To see viruses, one needs a very sophisticated type of electron microscope.

Unlike other microorganisms, viruses are not independent living creatures. They have no cell of their own and cannot replicate (multiply) on their own. To do that, they must enter into a host cell. They then program that cell such that the cell will forget its own task and will now concentrate on producing viruses. One can think of this being like a computer virus. The latter can multiply only when it has entered into another computer and reprogrammed it.

Viruses, like bacteria, are to be found in liquids and air, especially in cells. Outside a cell they can only survive for a limited period of time.

**Viruses are very resistant to cold, but heat quickly kills them**

Examples of viral diseases: influenza, hepatitis B, polio and AIDS

- Viruspartikel = virus particle
- DNA
- Proteinhülle bleibt draußen = protein coat remains on the outside
- Virale DNA drängt ein = viral DNA penetrates

- Viruses are not living creatures in the strict biological classification sense, since they cannot replicate without the help of another cell.
- Viruses are – to put it simply – like "cell pirates": they penetrate into cells and program them to do their bidding.
Some viruses cause harmless diseases in humans, but they also cause diseases that can lead to death e.g. AIDS.

1.1.4 Fungi

Fungi differ from bacteria especially in terms of their size. Fungi are much bigger than bacteria. Moreover, fungi have a structure that is different from that of bacterial cells. Biologists would say that they have a real cell nucleus.

Fungi are found worldwide and obtain their nutrients by breaking down dead organic materials (e.g. timber) or as parasites on plants, animals and people.

Of the more than 100,000 species of fungi, only very few can cause human diseases (e.g. foot, nail and skin fungi). But by producing toxins, fungi can also spoil foodstuffs.

Some fungi are even very useful for humans, for example the antibiotic penicillin is obtained from the mould *Penicillium*.

**Yeast:** yeasts also belong to the fungi.

For humans they are, on the one hand, of economic importance (e.g. sour dough, beer brewing) but they can also cause disease (generally in persons with a weak immune system).

The yeast that is best known in the medical setting is *Candida albicans*, the **thrush fungus**. This fungus often colonizes the nose, throat and mouth regions and lives there without causing disease, but it can also cause thrush infections (“oral thrush”, “nappy thrush”) (in particular in infants).

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Some fungi cause disease, whereas others are beneficial

**Penicillin** can be obtained from the mould *Penicillium*

1.1.5 Protozoa

In the animal kingdom a distinction is made between unicellular and multicellular organisms. Protozoa (singular: protozoan) are unicellular organisms that are to be found virtually everywhere where there is water. They are an important constituent of plankton and live in all habitats that are sufficiently moist.
We are familiar with e.g.

Flagellates, Rhizopods, Ciliates, Amoebae

Some protozoa can cause disease, these include African sleeping sickness, amoebic dysentery or malaria, i.e. many of the tropical diseases.

- Protozoa live in our waters
- They are important as pathogens, in particular, in the warmer regions of the world

1.1.6 Prions

Prions are, strictly speaking, not microorganisms but rather infectious proteins. Prions are known to be the causative organisms of “transmissible spongiform encephalopathies” (TSEs) (these are diseases where the brain takes on a sponge-like appearance). Examples of these diseases are “mad cow disease” (scientific term: bovine spongiform encephalopathy - BSE) in cattle, scrapie in sheep and Creutzfeld-Jakob disease (CJD) in humans. The disease triggered through ingestion (eating) of the causative organisms of BSE represents a new variant of CJD (=vCJD).

CJD is found worldwide at an annual rate of around 1-2 cases per million inhabitants and is characterized by its long incubation period (the interval between becoming infected and onset of disease), short clinical course and always fatal outcome. CJD patients suffer from various neuropsychiatric symptoms ranging from e.g. dementia to complete mental breakdown.

The pathogenic agents appear to be “abnormally conformed” (folded) forms of normal proteins (prions) in the central nervous system. One can imagine these to be like an umbrella that is turned inside out. In general, it offers us a good service but if it turns inside out we find ourselves standing there in the rain without protection (see figure). Similarly, proteins offer a good service but if they become abnormally folded they harm us. Prions are particularly resistant to physical (temperature) and chemical influences. Studies carried out so far indicate that prions stick very strongly to metal surfaces. Such properties make it much harder to clean and disinfect instruments contaminated with prions. This is why special decontamination
measures are needed, especially for the instruments used in neurosurgery and ophthalmology if CJD is suspected.

2 The science of infection

2.5 How are infections caused?

Infection: pathogens enter into the human body and multiply

For example, droplet infection: this method of infection is very common for the viruses that cause the “common cold”. The viruses are spread through tiny droplets composed of nasal secretions, containing millions of viruses. When somebody sneezes or blows their nose, the viruses are expelled with the droplets from the nose into the air and breathed in by other people. Now they are able to colonize the mucous membranes of another victim’s nose.

A prerequisite for infection is that a pathogen (bacteria, viruses, fungi, protozoa) be present. One single bacterium cannot cause disease; the starting point is always the infection source. In the infection source the pathogens multiply. For example, just like water pours forth from a spring, so the pathogens pour forth from the infection source.

The pathogen must reach its infection target (e.g. the human being) through transmission.

This can occur directly through contact or droplets or indirectly e.g. through hands, clothing, air, etc.
2.5.1 Basic model for transmission of infectious diseases:

<table>
<thead>
<tr>
<th>Infection source</th>
<th>Transmission route</th>
<th>Infection target</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pathogen</em></td>
<td><em>direct, indirect</em></td>
<td><em>Patient</em></td>
</tr>
</tbody>
</table>

**Living (animate):**
- Patient, staff member

**Non-living (inanimate):**
- Drugs, water, Food, etc.
- Objects
- Surfaces
- Air

**Factors:**
- Hands
- Objects
- Surfaces
- Air
- Immune system
- Age
- Underlying disease
- Interventions

**Nosocomial infection (=hospital acquired infection):** Infection that a patient contracts in hospital

The main difference between infections contracted outside the hospital and nosocomial infections is that the patient is, because of his/her underlying disease, generally much more susceptible to infection than is a healthy person.
2.6 *Infection sources*

INFECTION SOURCE: Starting point of a pathogen, which multiplies there before it is spread. This starting point can be either the human being himself or it can be an external source, either living or non-living.

The most important infection sources are:
The human being with his/her own microorganisms (intestines, vagina, skin, infection foci) is the most important living infection source, e.g. urinary tract infections caused by one’s own intestinal bacteria or scarlet fever through droplet infection.

**Carriers**: are persons who excrete (shed) pathogens – without having been ill themselves.

**Chronic excretors**: are persons who after suffering from an infection become well again but continue to excrete pathogens (e.g. after a salmonella infection).

In particular in the case of nosocomial infections, hospital staff, as carriers, are an important infection source e.g. *Staphylococcus aureus* (pathogen causing pus) is often found in the nose.

**Animals**: animals can also be an infection source: e.g. rabies infection arising from a bite injury or salmonella infection from inadequately cooked chicken.
**Water**: is an infection source because of the typical water bacteria it contains such as legionellae or pseudomonads and also because of the contaminating microorganisms that enter the water (e.g. typhoid fever bacteria through faecal contamination).

**Food**: several microorganisms can multiply in foodstuffs; foodstuffs represent infection sources for bacterial infections (e.g. salmonella infections due to inadequately heated dishes containing eggs).

---

**AND YOU?**

**BLACKMAIL? ROBBERY?**

**MANSLAUGHTER?**

**SALMONELLA!**
2.7 Infection routes

The patient may be his/her own infection source and spread the pathogens, e.g. through a smear infection to wounds.

Hospital personnel are often responsible for smear infection, if they do not discharge medical / nursing duties with sufficient technical know-how and care.

Direct transmission routes indicate direct contact between the person and the infection source (example: a surgeon carries out surgery while one of his fingers oozes pus). Besides, a droplet infection can give rise to direct transmission (example: coughing, sneezing).

Indirect transmission of pathogens occurs e.g. via hands.
Example: inadequate hand hygiene after going to the toilet.

Of the numerous transmission routes that are possible in the hospital, the hands of healthcare workers are the most important.

The following applies for microbial transmission in general:
The less often objects come into contact with the patient, and the farther they are from him/her, the lesser will be the infection risk posed by them.
2.8 Pathogens causing hospital infections

The most common pathogens causing hospital infections are *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

*Escherichia coli* (an intestinal bacterium): the most common pathogen causing urinary tract infections.

*Staphylococcus aureus* (a bacterium found on the skin and mucous membranes): a typical cause of pus and thus the most common pathogen causing wound and skin infections.

*Pseudomonas aeruginosa* (bacterium found in humid environments): one of the most common pathogens causing lung infections in intensive care units.

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