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Antimicrobial Agents

Course Handout

Third-year Bachelor's degree in Microbiology

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Ref……

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Abbreviations list

Abbreviation	Full Term
5-FC	Flucytosine
AACs	Chloramphenicol acetyltransferases
AGE's	Aminoglycoside-modifying enzymes
D	Decimal reduction time
DNA	Desoxyribonucleic acid
HIV	Human Immuno deficiency Virus
HPP	High Pressure Processing
MBC	Minimum bactericidal concentration
MIC	Minimum inhibitory concentration
NAG	N-acetylglucosamine
NAM	N-acetylmuramic acid
PBP	Penicillin-binding proteins
RNA	Ribonuleic acid
TDT	Thermal Death Time
Tmax	Maximum temperature
Tmin	Minimum temperature
Topt	Optimum temperature
UHT	Ultra High Temperature
UV	Ultraviolet

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Antimicrobial agents constitute a fundamental component in the prevention and treatment of infections caused by various microorganisms, including bacteria, viruses, fungi, and parasites. Since the discovery of penicillin, these agents have profoundly transformed medical practice by significantly reducing the morbidity and mortality associated with infectious diseases. They comprise a range of natural or synthetic substances capable of inhibiting the growth of microorganisms or destroying them. However, the emergence of antimicrobial resistance represents a major public health concern, requiring the rational use of these agents and a better understanding of their mechanisms of action.

This course handout aims to present, in a progressive and structured manner, the different aspects related to antimicrobial agents. It is organized into six chapters.

Chapter 1 is devoted to fundamental concepts. It introduces essential definitions such as sterilization, asepsis, and disinfection, as well as evaluation tools such as the antibiogram, the minimum inhibitory concentration (MIC), and the minimum bactericidal concentration (MBC).

Chapter 2 deals with physical agents used in the control of microorganisms, including heat, radiation, and filtration. Chapter 3 addresses chemical agents, particularly antiseptics and disinfectants, as well as their modes of action and conditions of use.

Chapter 4 is dedicated to antibiotics, discussing their mechanisms of action, their sites of action, and the resistance mechanisms developed by bacteria.

Chapter 5 presents antifungal agents, detailing their modes of action, their targets, and the resistance phenomena observed in fungi.

Finally, Chapter 6 focuses on antiviral agents, emphasizing their mechanisms of action, their sites of intervention, and the resistance mechanisms of viruses

Thus, this subject enables students to acquire essential knowledge to understand and master the different strategies for controlling microorganisms, while providing the necessary theoretical foundations for understanding antimicrobial agents and highlighting their importance in current biomedical practice, as well as the challenges associated with their use.

1. Definitions and concepts

1.1. Antimicrobial agent

Antimicrobial agents are substances or processes capable of destroying microorganisms (microbicidal effect) or inhibiting their growth and multiplication (microbiostatic effect). They act on various types of microorganisms, such as bacteria, viruses, fungi, and some parasites, and are used in fields such as medicine, hygiene, and industry.

1.2. Action of antimicrobial agents

Antimicrobial agents work either by inhibiting the growth of microorganisms or by destroying them.

A specific suffix is used to indicate the type of action of the antimicrobial agent:

- Substances that destroy organisms (lethal treatment) often have the suffix **-cide** (from Latin *coedere*, to kill). Their action is lethal:
  - **Bactericide**: kills bacteria, except possibly bacterial spores (endospores)
  - **Sporicide or sporulicide**: kills bacterial spores (endospores)
  - **Fungicide**: kills fungi (including their spores, though these spores do not have the same resistance properties as bacterial endospores)
  - **Viricide or virulicide**: inactivates viruses
- Other substances do not kill but prevent the development of microorganisms (stabilizing treatment). Their names end with the suffix **-static** (from Greek *statikos*), such as:
  - **Bacteriostatic**: temporarily inhibits bacterial growth
  - **Fungistatic**: temporarily inhibits fungal mycelial growth

Some agents may exhibit both modes of action depending on the dose.

1.3. Spectrum of action

The spectrum of action of an antimicrobial agent refers to the range of microorganisms on which the agent or process exerts a biostatic (growth-inhibiting) or biocidal (microbe-killing) effect. In other words, it is the “range” of bacteria, viruses, fungi, or other microorganisms that the agent can affect.

The spectrum of action can be classified according to its breadth:

- **Very broad**: The agent acts on many different types of microorganisms. For example, some broad-spectrum antibiotics can kill both Gram-positive and Gram-negative bacteria.
- **Broad**: The agent affects a large number of microorganisms, but not all.
- **Medium**: The agent is active against a limited group of organisms.

- **Narrow**: The agent specifically targets one type or a small group of microorganisms.

The spectrum of action is important because it guides the choice of the agent or process depending on the goal:

- **Wide prevention**: When the aim is to reduce general contamination.
- **Targeted action**: When the specific microbe is known and there is a desire to avoid affecting other organisms.

1.4.Conditions affecting the effectiveness of antimicrobial agents

The effectiveness of antimicrobial agents depends on several factors related both to the agent itself and to the conditions in which it is used. Understanding these conditions is essential to optimize their bactericidal or bacteriostatic power and to ensure effective disinfection or sterilization.

- **Population size**

It takes more time to destroy a large population than a small one.

- **Composition of the population**

The effectiveness of an agent varies with the type of organism being treated, as microorganisms differ greatly in their susceptibility.

- **Concentration or intensity of an antimicrobial agent**

Generally, a higher concentration kills more quickly (although this relationship is not linear).

- **Exposure time**

Longer exposure results in more organisms being killed.

- **Temperature**

An increase in temperature enhances the agent's activity.

- **Local environment**

Several factors, such as pH and the concentration of organic matter, can also influence effectiveness.

1.5.Definitions

1.5.1. Sterilization

Sterilization is a process aimed at killing all microorganisms in a preparation. The treated material is considered sterile when no microorganism is capable of growing. A product or material is said to be sterile if no germ is revivable or capable of growth (irreversible loss of reproductive capacity). The product must be kept in packaging that prevents recontamination. When applied to inanimate objects, sterilization agents can be physical or chemical.

1.5.2. Disinfection

Disinfection is the destruction, inhibition, or removal of potentially pathogenic microorganisms and also substantially reduces the total microbial population. A disinfectant does not necessarily sterilize an object, as viable spores (bacterial endospores) may remain. Unlike sterilization, the effect of disinfection is temporary.

1.5.3. Disinfectants

Disinfectants are chemical agents used only on inanimate objects. Therefore, they can be used in high concentrations and for prolonged contact times. They can be in the form of vapors, liquids, or solids.

1.5.4. Antisepsis

(from Greek *anti*, against, and *sepsis*, putrefaction) a temporary operation aimed at destroying or inhibiting microorganisms and/or inhibiting viral activity on living tissues according to their tolerance threshold.

1.5.5. Antiseptics

Antiseptics are chemical products whose action is limited to the species on which they are active. They act on living tissues within the limits of their tolerance, e.g., iodine tincture, mercuric laurate, potassium permanganate, hydrogen peroxide, etc. Since they must not damage host tissue, antiseptics are generally less toxic than disinfectants. Their action is almost immediate but not long-lasting.

1.5.6. Asepsis

Asepsis refers to all measures preventing any exogenous introduction of microorganisms. Sterilization and disinfection are means to achieve asepsis.

- **Aseptic**: a term describing an environment free of microorganisms
- **Septic**: a term describing an environment containing microorganisms
  A product is considered non-sterile or septic if it contains microorganisms.

1.6. Measurement of antimicrobial activity

1.6.1. Physical agents

To evaluate the effectiveness of physical antimicrobial agents, several concepts help quantify microbial resistance and optimize treatments. The decimal reduction time (D) corresponds to the time required to reduce 90% of the microbial population under given conditions. In other words, if a sample is exposed to a specific temperature or radiation dose, D represents the duration needed for only 10% of the initial microorganisms to remain viable. This parameter is

particularly useful for comparing the sensitivity of different microbial species to the same physical treatment and for planning effective sterilization processes. The Z value, on the other hand, indicates the temperature change required to alter the decimal reduction time by a factor of 10. It thus allows the relative thermal resistance of a microorganism to be assessed: the higher the Z value, the more the microbe can tolerate temperature variations before the treatment becomes effective. Together, these two parameters provide a precise framework for determining not only the optimal duration and intensity of the treatment but also for adapting protocols according to the type and initial quantity of microorganisms present. Thus, unlike chemical agents whose effectiveness depends on concentration, physical agents require an approach based on exposure time, treatment intensity, and the specific response of the microbes to ensure reliable and reproducible microbial destruction.

1.6.2. Chemical agents

The activity of chemical antimicrobial agents is measured by their ability to inhibit or destroy microorganisms depending on their concentration. This evaluation relies on standardized methods such as the antibiogram, which studies microbial sensitivity, as well as determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Another tool is the phenol coefficient. These parameters are essential for selecting the most effective chemical agent and adjusting its use according to the microorganism type and application conditions.

1.6.2.1. Phenol coefficient

The phenol coefficient is a measurement used in microbiology to evaluate the effectiveness of a disinfectant by comparing it to that of phenol. It is defined as the ratio between the dilution of the tested disinfectant and that of phenol that produce the same bactericidal effect on a given bacterial strain under identical experimental conditions of time and temperature. This allows for the quantification and comparison of the bactericidal power of disinfectants.
Consequently:

- **Coefficient > 1**: the disinfectant is more effective than phenol
- **Coefficient = 1**: effectiveness equivalent to phenol
- **Coefficient < 1**: the disinfectant is less effective than phenol

1.6.2.2. Antibiogram

An antibiogram is a laboratory method used to determine a bacterium's sensitivity to different antibiotics to identify the most effective treatment. The principle involves exposing an isolated bacterium to several antibiotics on an appropriate culture medium. After incubation, bacterial growth is observed around the antibiotic disks or solutions. The formation of a zone of inhibition (Figure 1) indicates that the antibiotic prevents bacterial multiplication, allowing the bacterium to be classified as sensitive, intermediate, or resistant to the tested treatment.

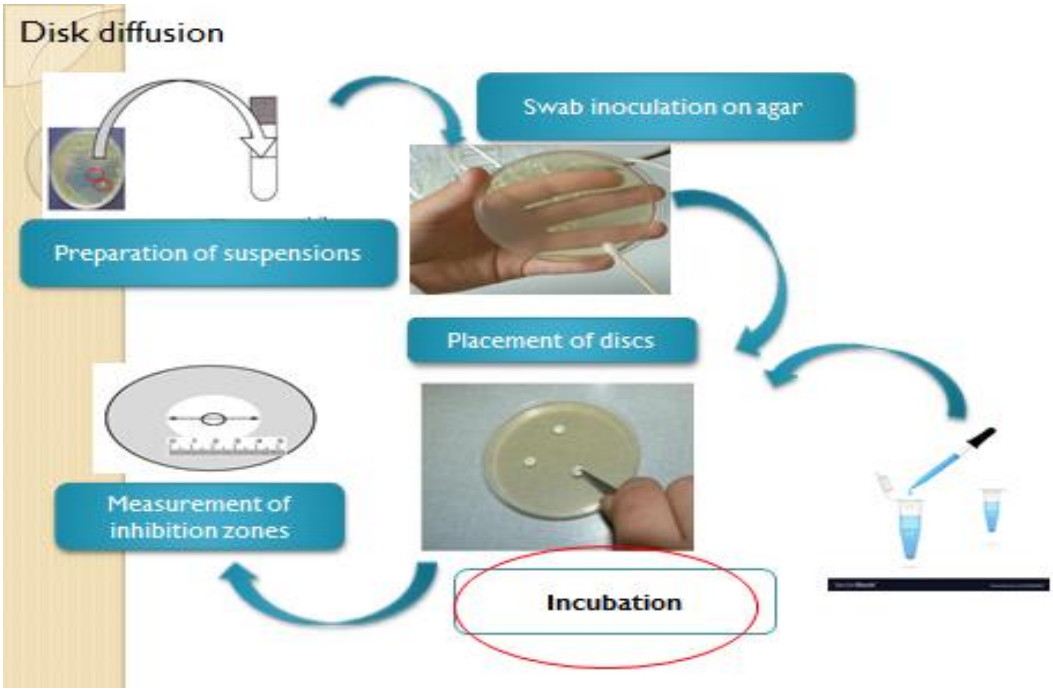


Figure 1. Steps for performing an antibiogram

1.6.2.3. Minimum Inhibitory Concentration

The Minimum Inhibitory Concentration (MIC) is defined as the minimum concentration of an antibiotic or chemical agent that inhibits the *in vitro* growth of 99% of the tested microbial population. In practice, the MIC is the lowest concentration, within a series of twofold dilutions of the antimicrobial agent, that results in the inhibition of all visible microbial growth. There are several techniques used to determine the minimum inhibitory concentration (MIC).

A. Dilution method

Dilution methods can be performed in liquid or solid media. They involve exposing a standardized bacterial inoculum to increasing concentrations of antibiotics according to a

geometric progression with a ratio of 2. After incubation, the MIC is indicated by the tube or well containing the lowest concentration of antibiotic in which no growth is visible (Figure 2).

In solid media, the antibiotic or antimicrobial agent is incorporated into a gelled medium poured into Petri dishes. The surface of the agar is inoculated with the strains to be tested. After incubation, the MIC for each strain is determined by the inhibition of growth on the medium containing the lowest concentration of the antibiotic.

B. E-test method

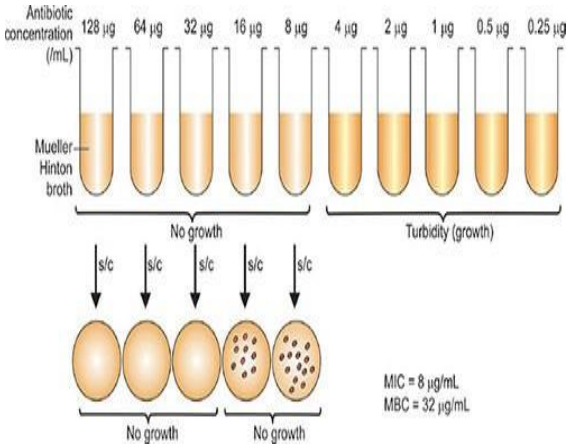
Determining the MIC using classical methods is often difficult to apply in routine practice. The commercialization of a rapid and simple technique, the E-test, allows a laboratory to obtain an indirect estimation of the MIC.

The E-test, a method performed on solid (agar) media, enables MIC determination using strips impregnated with a continuous exponential gradient of the antibiotic to be tested (Figure 2). Depending on the molecule, this gradient covers a range from 0.016 to 256 mg/L or from 0.002 to 32 mg/L.

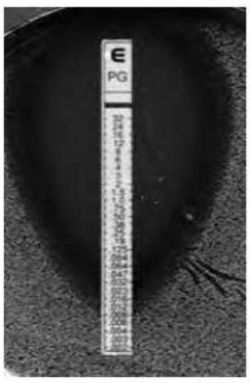
The principle of the E-test combines characteristics of both diffusion and dilution methods on solid media. The strips (inert, hydrophobic supports, 5 mm wide and 50 mm long) are placed on the surface of an agar medium previously inoculated with the strain to be tested.
After incubation, microbial growth inhibition appears as an ellipse of inhibition, and the point where the ellipse intersects the strip indicates the MIC. A reading scale printed on the strip allows for rapid interpretation.

1.6.2.4.Minimum Bactericidal Concentration

The Minimum Bactericidal Concentration (MBC) is the lowest concentration of an antimicrobial agent that leaves no more than 0.01% of the original inoculum surviving (i.e., 1 survivor out of 10,000). To determine the MBC, bacteria from the tubes showing no growth during the MIC determination are collected and inoculated onto an antibiotic-free medium. The lowest concentration of the antimicrobial agent that prevents any bacterial regrowth is considered the MBC (Figure 2).



MIC and MBC determination



E-test

Figure 2. Determination of MIC and MBC by the dilution method and illustration of the E-test with an antibiotic strip (Pankey et al, 2013).

1.7. Examples of application

Question 01:

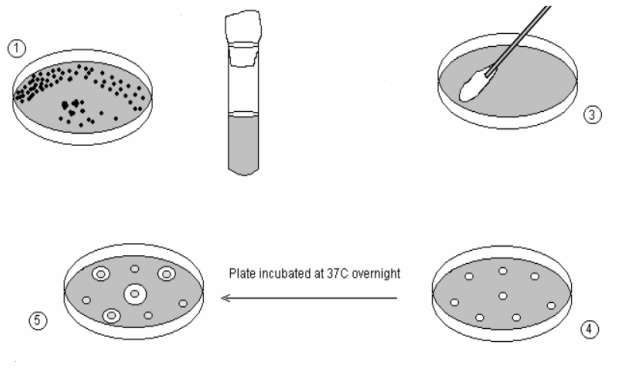
There are different methods to prevent microorganisms from entering or growing in the human body: cooking food, disinfecting a wound, and washing hands before eating. Which of these are asepsis? Which are antisepsis?

Asepsis: ……

Antisepsis: ……

Question 02 :

Observe the figure:



1. What does the figure represent?
2. Specify the five steps mentioned in the figure.

Question 03:

In the laboratory, the stock solution of this drug is 200 µg/mL. They prepare a series of successive dilutions in tubes, each containing 1 mL of broth. At each step, they take 0.5 mL from the previous solution and add 0.5 mL of broth. After incubation with a bacterium, they obtain the following results:

Tube	Bacterial growth	Antibiotic concentration
1	–	…….
2	–	…….
3	–	…….
4	+	…….
5	+	…….

9. Calculate the antibiotic concentration in each tube (to complete the table).
10. Determine the MIC.

2. Physical agents

Chapter 2 introduces physical agents used to control and eliminate microorganisms. These methods rely on physical factors such as temperature, radiation, and filtration to inhibit microbial growth or achieve sterilization. They are widely applied in medical, laboratory, and industrial settings due to their effectiveness and versatility. This chapter explores the different types of physical agents, their mechanisms of action, and their practical applications in ensuring hygiene and safety.

2.1. Thermal treatments

2.1.1. Effect of temperature on microbial growth

Temperature profoundly influences microbial multiplication as well as metabolism (thermal sensitivity of enzyme-catalyzed reactions). Microbial growth depends on so-called cardinal temperatures (Figure 3): minimum (Tmin), maximum (Tmax), and optimum (Topt) growth temperatures. The Topt, where growth capacity is at its maximum, is always closer to Tmax than Tmin.

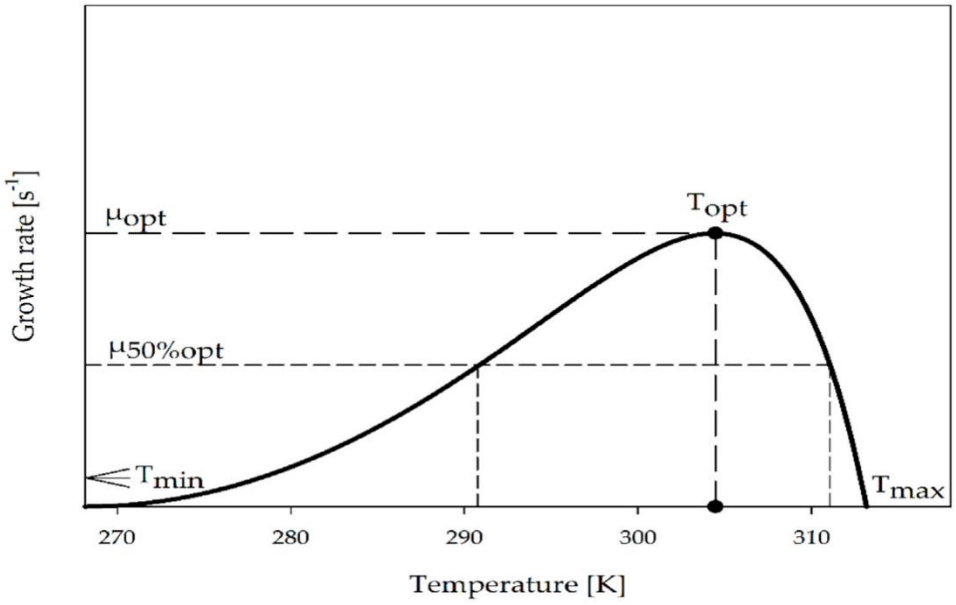


Figure 3. Effect of temperature on microbial growth (Noll et al, 2020).

Before the Topt, the growth capacity increases steadily with temperature. The rate of catalysis increases and approximately doubles with every 10°C rise in temperature. Global metabolism is activated, and the microorganism grows faster.
After the Topt, the growth capacity decreases due to the detrimental effects of temperature on

enzymes, transport systems, and other proteins. Temperature also has a significant effect on microbial membranes. At very low temperatures, membranes solidify. At high temperatures, the lipid bilayer melts and disintegrates. At temperatures above Tmax, no growth occurs. However, microorganisms are not always killed. This depends on the temperature value and the microorganism.

2.1.2. Influence of temperature on microbial destruction

The effect of temperature on microorganisms depends on:

Temperature level: Higher temperatures generally speed up microbial destruction by denaturing proteins and disrupting cellular structures. However, temperatures above a certain threshold can kill microorganisms, while lower temperatures can merely inhibit growth.

Duration of exposure: The length of time the microorganism is exposed to the temperature is a critical factor. Short bursts of high temperature might not be sufficient to kill microbes, while prolonged exposure to lower temperatures can still be effective in killing or inhibiting growth.

Type of microorganism: Different microorganisms have varying levels of heat resistance. Spores, for example, are much more heat-resistant than vegetative cells. Some microorganisms, such as *Clostridium* and *Bacillus* species, are especially resilient to high temperatures.

Medium composition: The nature of the environment where the microorganisms are found (e.g., the presence of water, nutrients, or other substances) can influence how temperature affects microbial survival. For example, certain additives like water or nitrites may enhance the effectiveness of heat treatment.

Microbial form: Vegetative cells, spores, and different microbial life stages respond to temperature in varying ways. Spores are generally more heat-resistant, requiring higher temperatures or longer exposure times to be destroyed.

2.1.3. Heat sterilization processes

Several methods can be used depending on the needs.

2.1.3.1. Processes using dry heat

A. Flaming

Flaming consists of slowly passing the objects to be sterilized through the blue flame of a Bunsen burner in order to destroy any organic material present on their surface. The Bunsen burner is a gas-powered device, invented in 1855 by Peter Desdega, but it is named after the German physical chemist Robert Bunsen (1811-1899). This technique is specifically designed for glass objects (such as the tips of pipettes, glass rods, and the necks of test tubes) and certain

metal objects (such as handles and forceps). It is important to note that the blue flame, being the hottest part of the flame, creates a sterilized zone with a diameter of approximately 20 cm, due to the rising air within that zone. All manipulations involving the opening of tubes and culture dishes, as well as inoculations, should be performed within this sterilized zone. However, it is recommended not to move the Bunsen burner after it has been lit and during manipulation.

B. Pasteur oven

The Pasteur oven is a dry heat incubator that circulates hot air inside the chamber to evenly distribute the heat (Figure 4). It is used for sterilizing empty glassware (such as test tubes, Petri dishes, culture tubes and caps, graduated pipettes, and various containers), porcelain, and metal instruments. It is important to note that the objects to be sterilized must be clean and thoroughly dry. They may need to be plugged with sterile cotton and wrapped in sturdy paper or aluminum foil. The items are then placed inside the oven and heated for specific periods: 4 hours at 140°C, 2.5 hours at 160°C, or 30 minutes at 180°C.

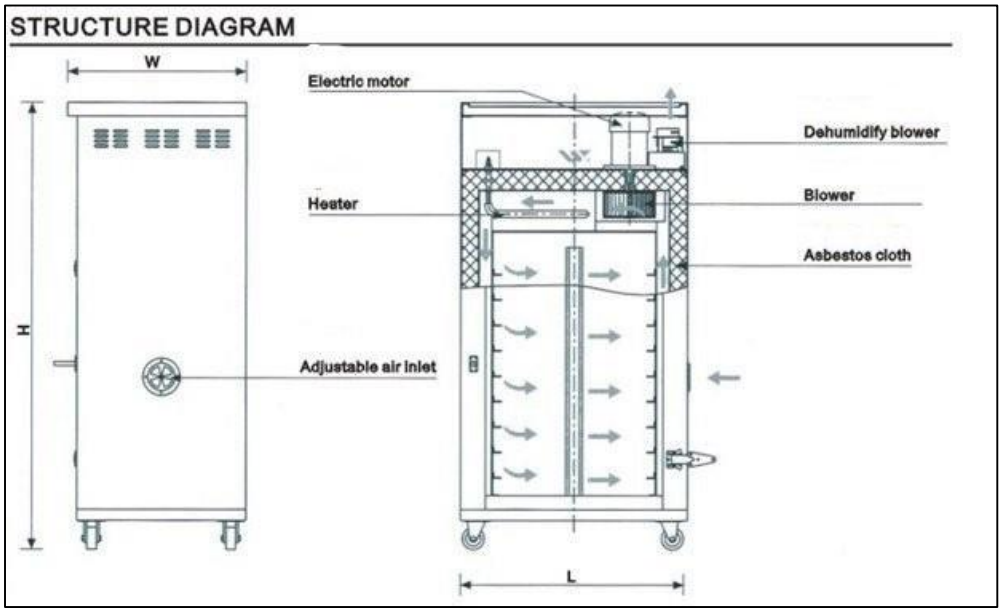


Figure 4. Pasteur oven (Alkadhim, 2018).

2.1.3.2. Processes using moist heat

A. Autoclave sterilization

It is a device that exposes the material to be sterilized to the action of steam, free of air and under pressure, at temperatures ranging from 115 to 140 °C for a specified time (Figure 5). In

general, bacteria and spores are destroyed within 15 minutes at 120 °C under a pressure of 2 bars. Moreover, autoclaving occurs at lower temperatures than those used for dry heat sterilization, which is explained by the solubilization effect that accompanies the thermal action of the autoclave. The material to be sterilized is prepared beforehand; for example, glassware is plugged with cotton and wrapped in aluminum foil or very strong wrapping paper. In addition, containers with culture media are filled up to three-quarters (whether new or contaminated), while metal instruments are placed on trays of special boxes containing a 2 % sodium carbonate solution to prevent rust. However, metal instruments are usually sterilized by dry heat rather than by autoclaving. The autoclave is also used for sterilizing media containing biological products. On the other hand, not all media can be sterilized using this method, such as those containing delicate substances like gelatin, casein, albumin, etc., which can denature. It should be noted that the material to be sterilized is placed in the metal baskets of the autoclave, and the water level is checked before each sterilization operation (it must not exceed the basket support).

Figure 5. Example of a standard benchtop autoclave (Fund, 2018).

B. Water Bath

In microbiology, a water bath can be used as a sterilization tool (Figure 6). Heating for 10 minutes at 80 °C is sufficient to destroy all vegetative forms of bacteria. However, spores are

not destroyed under these conditions. The effectiveness of boiling can be improved by adding concentrated saline solutions to the water (raising the boiling point) or by prolonging the heating. This method can be used for the sterilization of delicate liquid products, such as: albumin-based media, milk, gelatin, concentrated sugar solutions, etc.

Figure 6. Example of a laboratory Water Bath (Fund, 2018).

C. Tyndallization

The tyndallization process**,** described by THYNDALL, requires a relatively low temperature of 60 °C to 70 °C. The heating duration should be 30 minutes to 1 hour, repeated three times, allowing an interval of 12 to 24 hours at room temperature between each heating (Figure 7). During heating, only the vegetative forms are inactivated, while during the intervals, most thermoresistant spores germinate and become sensitive to the next heating. This allows the destruction of spores without using excessively high temperatures**.** This method is used for delicate media containing serum, eggs, or any thermosensitive, highly viscous substance that cannot be sterilized by autoclaving or filtration.

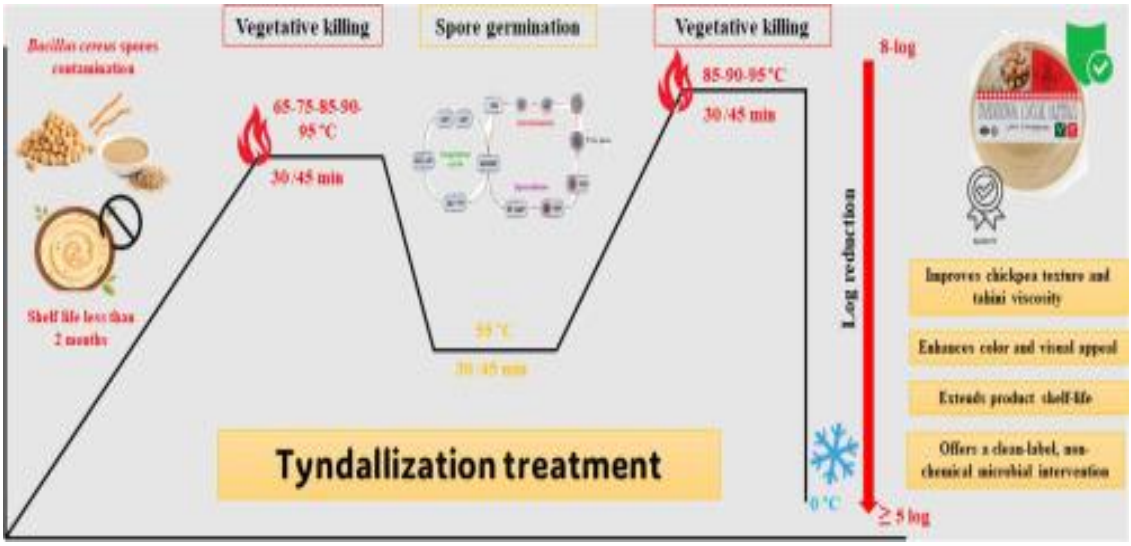


Figure 7. The tyndallization process (Daoud et al., 2025).

D. Pasteurization

This method was developed in 1865 by the French scientist Louis Pasteur (1822-1895). It is used to preserve foods naturally for a limited time by eliminating vegetative microorganisms while leaving spores unaffected. There are several types of pasteurization depending on the temperature and duration. In low-temperature pasteurization, the product is kept at 60-65 °C for at least 30 minutes in a double-walled chamber containing hot water, as in the pasteurization of fruit juices. High-temperature pasteurization involves heating the product at a higher temperature (70-90 °C) for a very short time (15 seconds to 2 minutes) using plate or tubular heat exchangers, commonly applied to milk and vegetable purees. Ultra High Temperature (UHT) sterilization heats the product in a thin layer at 138-140 °C for a few seconds**,** destroying all vegetative forms and spores. This method is very sensitive and carries a high risk of product denaturation. Pasteurization is always followed by rapid cooling. It can be carried out in bottles or in bulk (Figure 8).

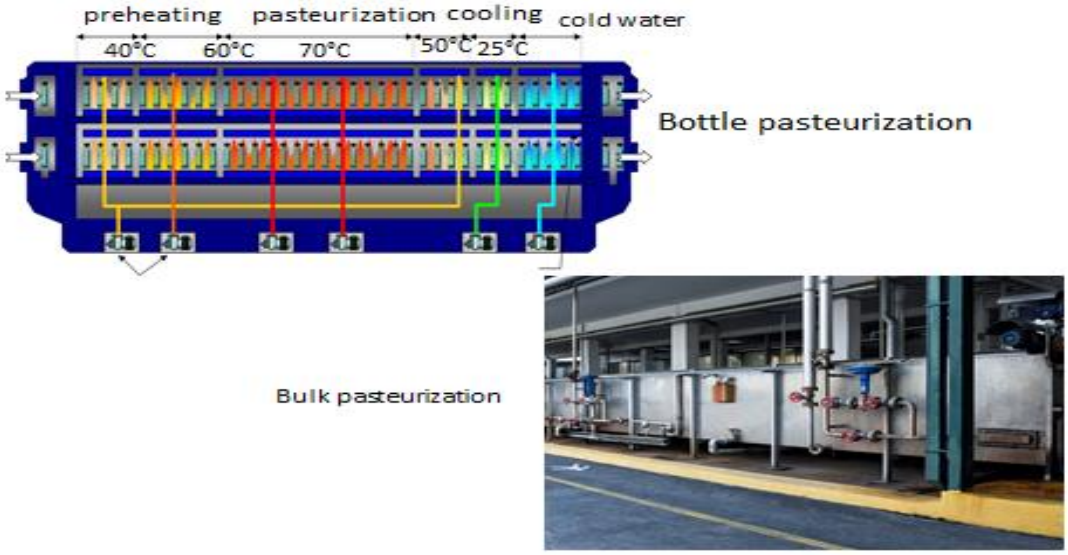


Figure 8. Bottle and bulk pasteurization (Ayers, 2015).

2.1.3.3. Other thermal sterilization processes

A. Thermization

It is a mild heat treatment method, mainly used for milk. It involves heating the product to a temperature below that of pasteurization, usually between 57 and 68 °C for 15 to 30 seconds, to reduce the microbial load while preserving the product's sensory and nutritional qualities. Unlike pasteurization, thermization does not eliminate all pathogenic microorganisms, so the product must be kept refrigerated and consumed quickly.

B. Blanching

It is a brief heat treatment mainly applied to vegetables and fruits before preservation, such as freezing or canning. It involves immersing the product in hot water or steam for a few minutes, followed by rapid cooling. This process reduces the surface microbial load, eliminating or inhibiting some bacteria, yeasts, and molds, while not destroying spores, meaning the product is not sterile. Blanching also inactivates enzymes responsible for deterioration, thus preserving the color, texture, and flavor of the food. The temperatures used vary depending on the method: 70-100 °C for hot water for 1 to 5 minutes, and 85-100 °C for steam, usually for a slightly shorter time. Rapid cooling, in cold water or by cold air, is essential to stop the cooking process and maintain product quality.

C. Appertization

Appertization developed by the French scientist Nicolas Appert, is a heat treatment used for the long-term preservation of foods. It involves heating the product to high temperatures, typically between 110 and 130 °C, for a sufficient time to destroy all pathogenic microorganisms and spores. This process ensures complete sterilization, eliminating bacteria, yeasts, and molds, as well as the enzymes responsible for deterioration, allowing the product to remain stable at room temperature without refrigeration.

2.1.3.4. Evolution of the microbial population over time

The destruction of a microbial population under the effect of temperature is not instantaneous; it is an exponential phenomenon, the inverse of the growth process. Therefore, the law of thermal destruction is of a logarithmic nature**.** The Thermal Death Time (TDT) is the time required, at a given sterilization temperature, to achieve commercial sterility. When the same microbial population (or resistant spores) is exposed to different temperatures, it is observed that the higher the temperature, the faster the destruction occurs (Figure 9).

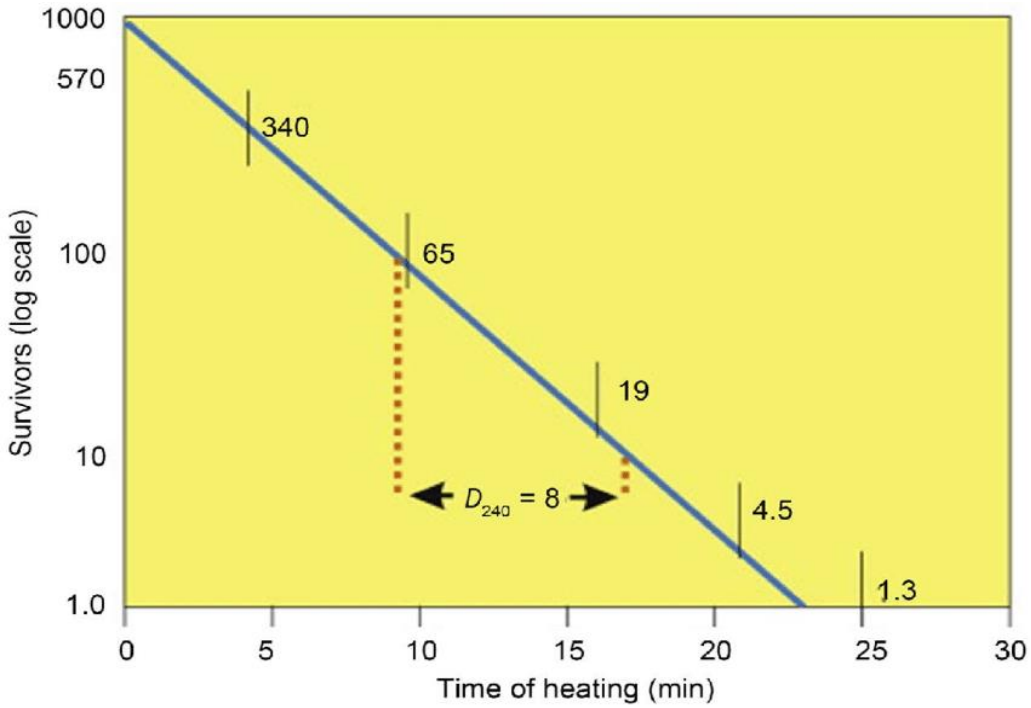


Figure 9. Thermal inactivation curve for a microorganism (Ghernaout and Elboughdiri, 2020). The D value is the period re- quested for demobilization of 90% of the microorganisms at a specific temperature. In this case, it needed 8 min to neutralize 90% of the microorganisms at 240˚F (115˚C) or D 240 = 8 min

The number of surviving germs decreases exponentially over time according to the following formula:

$$Log \frac{No}{N} = k \bullet t$$

N_0: initial population,

N: final population,

t: time,

k: constant, which varies depending on the type of microorganism and the sterilization temperature.

2.1.4. Cold stabilization processes

Cold stabilization processes significantly slow down or stop the growth of microorganisms without necessarily destroying them.

- **Refrigeration (0–10 °C):** slows bacterial and fungal multiplication, but some psychrotrophic microorganisms may continue to grow slowly.
- **Freezing and deep-freezing:** virtually halts all microbial activity by immobilizing the water available in the product, preventing the growth and reproduction of bacteria, yeasts, and molds.

2.2. Filtration

Filtration sterilization involves passing products through filters with pores measuring 0.45 µm**,** which retain most microorganisms except for viruses (≈0.22 µm). The fluid passes through the filter under pressure. Filters can be made from various materials, such as plastic, paper (cellulose), porcelain (Chamberland), glass, or metal. This technique is particularly suitable for heat-sensitive media**,** such as antimicrobial substances, vitamins, or sugar solutions, but it is effective only for low-viscosity liquids. Membrane filtration devices come in several types: for large volumes**,** funnel-type filters made of glass, plastic (polycarbonate), or metal (stainless steel) are used, while for small volumes**,** membranes are often integrated into syringe-adaptable filter cartridges, some of which are single-use and sterile, and others are autoclavable and reusable**.**

2.3. Centrifugation

Centrifugation is a physical method used as an indirect means of sterilization or clarification of liquids. It does not destroy microorganisms but allows the separation and removal of suspended particles**,** including some bacteria and yeasts, through centrifugal force. The liquid is subjected

to rapid rotation, causing the heavier particles to settle while the clarified liquid can be recovered. This technique is often used before or after other sterilization processes, such as filtration, to reduce microbial load or remove suspended matter, and it is particularly suitable for heat-sensitive media**,** such as sera, biological solutions, or certain fruit juices. However, it does not guarantee complete sterilization, as many microorganisms may remain in the clarified liquid.

2.4. Pressure

Pressure sterilization is a physical method that uses a combination of high pressure and, often, temperature to destroy microorganisms in foods or liquids. Depending on the process, it can be applied alone (high hydrostatic pressure) or in combination with heat (autoclaving).

- High Pressure Processing (HPP): the product is subjected to very high pressures (typically 400 to 600 MPa) for a few minutes. This pressure destroys bacteria, yeasts, and molds while preserving nutritional and sensory qualities, as the temperature remains relatively low.
- Autoclaving (pressure and heat): the product is heated to a high temperature under pressure (for example, 121 °C under 1 atm of additional pressure) to ensure complete sterilization, destroying all microbial forms, including spores.

2.5. Radiation

Radiation sterilization is a method used to eliminate all microorganisms (bacteria, viruses, fungi, and spores) by using high-energy radiation. It mainly involves gamma rays from Cobalt-60, X-rays, or ultraviolet (UV) rays. This technique is widely used to sterilize medical equipment, pharmaceutical products, certain foods, and even air or water. It offers several advantages, including high efficiency, the absence of heat (making it suitable for heat-sensitive materials), and the ability to treat already packaged products. However, it also has some drawbacks, such as high costs, strict safety requirements, and the potential alteration of certain properties of the treated products.

Ionizing radiation, such as beta and gamma rays, has a strong penetrating power in matter, which allows its use in the industrial sterilization of plastic materials like Petri dishes, pipettes, and flasks. Beta rays are accelerated electrons produced by an electrostatic accelerator and can pass through several tens of centimeters of solid material, whereas gamma rays, particularly those emitted by Cobalt-60, are electromagnetic waves with even greater penetrating ability. Their action is based on ionization, meaning the formation of ions by the removal of electrons from atoms, leading to molecular damage. In water (Figure 10), this process generates free

radicals that can attach to DNA and cause strand breaks, thereby affecting genetic information and potentially disrupting cell function.

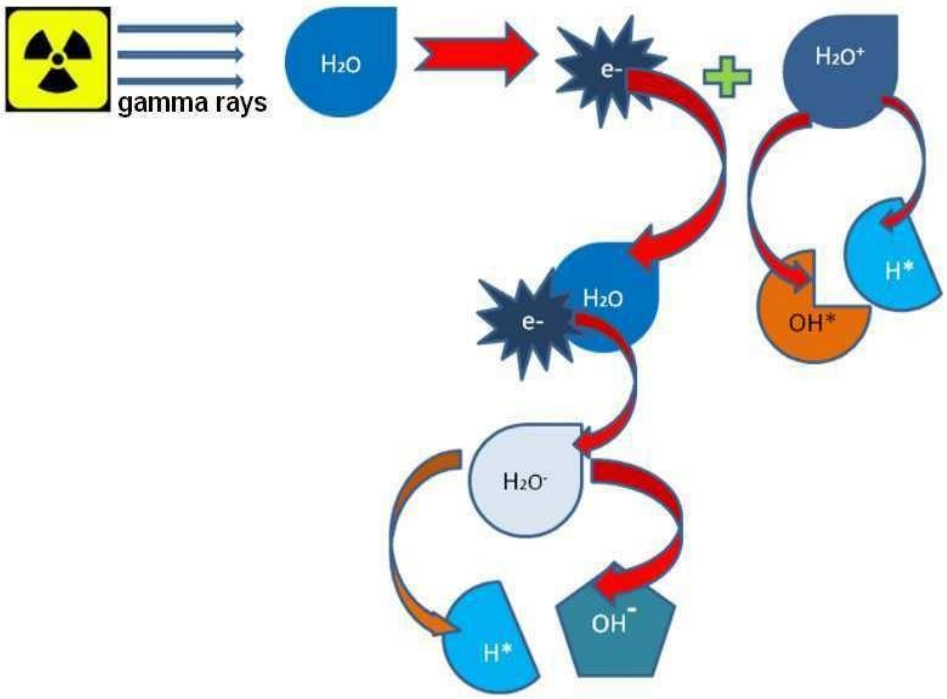


Figure 10. Effect of gamma rays on water molecules (da Silva Aquino, 2012).

Ultraviolet radiation is produced by quartz tubes containing mercury vapor. It is commonly used in germicidal lamps (or sterilizing lamps) to disinfect surfaces and ambient air, especially in specialized rooms or laminar flow hoods designed for sterile handling. In laboratories, this method is used to decontaminate air and work surfaces under protective hoods. It can also be used to sterilize certain instruments or containers, such as Petri dishes, as well as liquids in thin layers. UV radiation acts by inducing alterations in the genome, thereby blocking the biochemical processes required for the reproduction of microorganisms

2.6. Examples of application

Question 1:

Complete the following table by specifying, for each method: the temperature used, whether the process is batch or continuous, and whether it allows spore destruction.

Methode	Temperature	Batch	Spores destroyed
Pasteurization	……	……	……
Tyndallization	……	……	……
Stérilization	……	……	……
Appertization	……	……	……
UV	……	……	……
Ionizing radiation	……	……	……

Question 2:

Complete the following statements:

1. Filtration allows the …… of microorganisms. This concerns liquid products ……..
2. Cold is a food preservation technique that ……, the …… reactions and the …….. of microorganisms.
3. ……. is a physical food preservation technique. It consists of ……., partially or totally, the water contained in ……..
4. …… is a milder form of pasteurization. Its main objective is to destroy pathogenic bacteria that may be present in milk, without altering its technological characteristics as much.

Question 3:

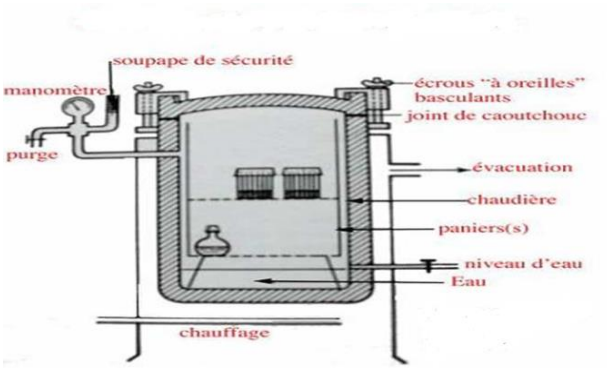


Figure 1

Figure 2

List the differences between the two processes corresponding to Figures 1 and 2

- Regarding time and temperature
- Regarding the objects sterilized
- Regarding the sterilizing agent and mechanism of action

3. Chemical agents

This chapter on chemical agents examines substances capable of neutralizing or inactivating microorganisms through specific biochemical reactions. Unlike physical methods, which act via external forces such as heat or radiation, chemical agents directly interfere with the cellular or molecular components of germs. Here, we will discuss the main categories of chemical agents, their mechanisms of action, as well as their uses in disinfection, antisepsis, and sterilization to protect health and ensure safety in various environments.

3.1. Definitions

Chemical substances are used as antimicrobial agents with a bactericidal effect. In general, they are referred to as disinfectants and antiseptics. The standard of the French Association for Standardization (AFNOR NF / March 1981) defines the terms antiseptic and disinfectant as follows:

3.1.1. Disinfectants

Disinfectants are substances or methods used to achieve disinfection under specific conditions. When their action is limited to certain microorganisms, this must be clearly specified. For example, a disinfectant that acts only on fungi is referred to as a fungicidal disinfectant.

3.1.2. Antiseptics

Antiseptics are substances used to perform antisepsis under specific conditions. When their action targets only certain microorganisms, this must be specified. For example, an antiseptic that is effective only against fungi is referred to as a fungicidal antiseptic.

3.1.3. Detergents

Detergents are chemical agents capable of solubilizing lipids and disrupting the integrity of cell membranes. Their action is based on their amphipathic nature, meaning they possess both a hydrophilic part (which attracts water) and a hydrophobic part (which attracts lipids), allowing them to interact with microbial membranes. By disturbing the lipid structure of membranes, detergents cause leakage of cellular components, compromising cell viability and potentially leading to cell lysis. These properties make detergents important agents for cleaning, disinfection, and antimicrobial action, particularly against bacteria and certain enveloped viruses.

3.2. Classification

Antiseptics, disinfectants, and detergents are essential agents for preventing infections and controlling microbial contamination. Although they share the common goal of reducing or eliminating microorganisms, they differ in their mode of action, usage, and spectrum of activity. Figure 11 provides a summary of their classification.

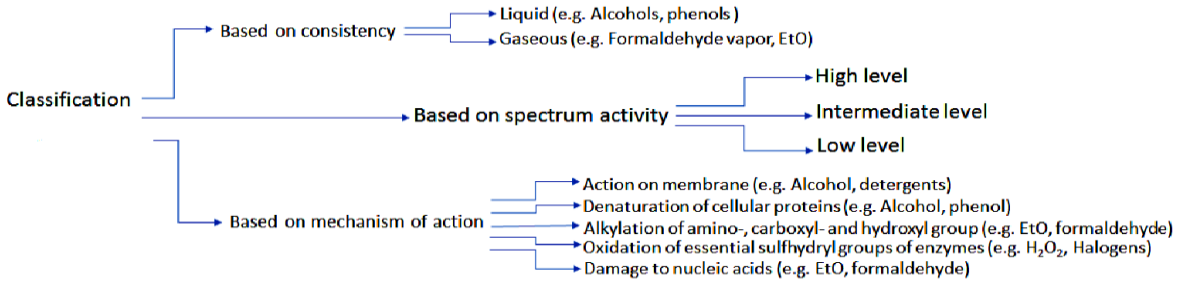


Figure 11. Classification of disinfectants (Yemiş and Harmancı., 2020).

3.3. Main families of antiseptics and disinfectant

3.3.1. Halogens

3.3.1.1. Chlorinated products

For over two centuries, chlorinated products have been used in industrial and medical settings for their bleaching, deodorizing, and disinfectant properties. Their use depends on concentration: at levels up to 5 chlorometric degrees, they can be applied as antiseptics on healthy skin, mucous membranes, and for wound irrigation, while at higher concentrations, they become irritating and are reserved for disinfection. Common solutions include bleach (0.016; 1.6; 1; 3 and 4° chlorometric), Dakin's solution (1.5° chlorometric), and Labarraque's solution (2° chlorometric), all composed of sodium hypochlorite and sodium chloride with an active chlorine content corresponding to the indicated concentration. Chlorine derivatives have a broad spectrum of activity, acting against bacteria (both vegetative and spore forms), fungi, viruses, and spores. Their action is rapid, starting within the first minute of contact, with their oxidizing power causing the destruction of membrane proteins.

3.3.1.2. Iodine-based products

Iodine products, made from iodine or iodine-containing compounds, are widely used today as antiseptics and disinfectants, with a lower risk of irritation and allergic reactions compared to pure iodine.

Iodine products include iodine and its derivatives, used in various forms. These include iodine alcoholic solutions (iodine alcohol, tincture of iodine) and aqueous iodine solutions (Lugol's solution, Tarnier's solution), the latter being rarely used today, even in gyneco-obstetrics, but

still employed for certain laboratory staining procedures. Iodophors are preparations that combine iodine or iodides with organic complexes, acting as “iodine carriers”: they increase the solubility, dispersion, and penetration of iodine, serve as a reservoir of iodine, and reduce the concentration of free molecular iodine. The most commonly used iodophor today is povidone-iodine (PVP-I)**.** Iodine products have a broad spectrum of activity, being bactericidal, virucidal, fungicidal, and sporicidal. Their action relies on the oxidizing power of molecular iodine, which quickly penetrates the cell membrane and destroys enzymatic and membrane proteins.

3.3.2. Alcohols

Alcohols, primarily ethyl alcohol at 60 or 70°, are used as antiseptics, either alone or as solvents to enhance the action of other antiseptics such as iodine or chlorhexidine. Available products include ethanol solutions of various alcohol concentrations prepared by dilution with water, such as modified 70° ethyl alcohol (camphorated) colored yellow (tartrazine), or 70° ethyl alcohol colored blue for pediatric use. These alcohols have a bactericidal spectrum of activity, are effective against *Mycobacterium tuberculosis*, have limited fungicidal activity, variable virucidal activity, and no sporicidal activity.

3.3.3. Biguanides

Biguanides are antiseptics generally used in the form of chlorhexidine digluconate or diacetate. They are available in different forms, including aqueous solutions such as 0.05% chlorhexidine and other products like Merfène (used for wound disinfection), Dosiseptine (for cutaneous application), and Hibidil (for the local treatment of skin conditions), as well as alcoholic solutions such as 0.5% alcoholic chlorhexidine, intended for local antisepsis. Biguanides have a bactericidal spectrum of activity against Gram-positive and Gram-negative bacteria, but they are less active against mycobacteria (except in alcoholic form), and they are neither sporicidal nor virucidal; acquired resistance has also been reported. Their mode of action depends on concentration: at low doses, they disrupt the cytoplasmic membrane, whereas at high doses, they cause precipitation of proteins and nucleic acids.

3.3.4. Quaternary ammonium compounds

Quaternary ammonium compounds are antiseptics generally used in combination with alcohol to enhance their effectiveness. The main compounds are benzalkonium chloride (a mixture of benzododecinium chloride and myristalkonium) and cetrimonium bromide. Their spectrum of activity varies depending on concentration: they are bactericidal or bacteriostatic, mainly

against Gram-positive bacteria, exhibit fungistatic activity, but are inactive against mycobacteria, have no sporicidal effect, and show weak activity against enveloped viruses (with no activity against non-enveloped viruses). Their mode of action involves several mechanisms, including partial denaturation of proteins and enzymes through solubilization and depolymerization, leading to enzymatic inactivation, binding to ribosomes with inhibition of protein synthesis, and disruption of the cell membrane, causing osmotic imbalance.

3.3.5. Oxidizing agents

Oxidizing agents are widely used as antiseptics and disinfectants due to their strong oxidizing power. They act by producing free radicals that damage essential components of microorganisms, such as proteins, lipids, and nucleic acids, leading to their destruction. Their action also involves the alteration of thiol (S–H) groups in certain amino acids of enzymes, disrupting enzymatic functions and resulting in cell death. The main oxidizing agents include hydrogen peroxide (H_2O_2), potassium permanganate, and peroxygen derivatives. Hydrogen peroxide, commonly used at about 3% in aqueous solution, is an effective antiseptic, although its use is limited by its rapid decomposition and the resistance of some catalase-producing microorganisms. These agents have a broad spectrum of activity, including bacteria, viruses, fungi, and sometimes spores depending on the concentration, and their effectiveness depends on factors such as concentration, contact time, and conditions of use

3.3.6. Aldehydes

Aldehydes are powerful chemical agents used as disinfectants, particularly in medical and hospital settings. They inactivate microorganisms by binding to their proteins and nucleic acids, thereby disrupting vital functions and leading to their destruction. Their broad spectrum of activity includes bacteria, viruses, fungi, and spores, making them especially suitable for disinfecting medical equipment, including heat-sensitive materials. Approximately 80% of aldehyde-based disinfectants are combined with quaternary ammonium compounds to enhance their effectiveness. The main products include formaldehyde, glutaraldehyde**,** and succinic aldehyde, although their use requires caution due to their toxicity and irritant properties.

3.3.7. Heavy metals

Heavy metals, such as mercury, silver, copper, arsenic, or lead, have historically been used as antimicrobial agents due to their toxic properties toward microorganisms. They act chemically on bacteria, fungi, or viruses to limit their growth or destroy them.

Their action relies on several main mechanisms:

- **Protein denaturation**: Metal ions bind to thiol groups (-SH) in microbial enzymes and proteins, altering their structure and blocking their biological functions.
- **Disruption of cell membranes**: Some metals disturb the integrity of bacterial or viral membranes, causing leakage of cellular contents.
- **Inhibition of metabolic enzymes**: Metals can replace essential metal ions in enzymes, inhibiting metabolic reactions necessary for the microorganism's survival.
- **Generation of oxidative stress**: Certain metal ions promote the production of free radicals, damaging microbial DNA, proteins, and membranes.

3.3.8. Other disinfectants and antiseptics

3.3.8.1. Carbanilides

Carbanilides are organic compounds derived from aniline, characterized by a diphenylurea-type structure, and are mainly used as antiseptic and antibacterial agents, particularly in skin hygiene products such as soaps and lotions; among the main representatives is Triclocarban, widely used for its activity against Gram-positive bacteria; their mechanism of action is based on the inhibition of bacterial growth through disruption of the cell membrane and interference with certain essential enzymatic functions, thereby limiting the proliferation of microorganisms on the skin.

3.3.8.2. Diamidines

Diamidines are organic compounds characterized by the presence of two amidine groups (-C(=NH)-NH_2), known for their antiseptic and antiparasitic properties, and mainly used in the treatment of certain skin and parasitic infections; among the main representatives are Pentamidine and Propamidine, used respectively against protozoa and as local antiseptics; their mechanism of action is based on their ability to bind to the DNA of microorganisms, thereby disrupting replication and nucleic acid synthesis, which prevents their multiplication.

3.3.8.3. Antiseptic dyes

Antiseptic dyes are organic compounds used topically for their antimicrobial properties, belonging to various chemical classes such as acridines, triphenylmethanes, and xanthene derivatives; they are applied for the disinfection of wounds, skin lesions, and mucous membranes; among the main representatives are methylene blue, gentian violet, acriflavine, and eosin; their mechanism of action involves interaction with microbial cellular components, particularly through binding to DNA and proteins and causing protein denaturation, thereby disrupting vital functions and inhibiting microbial growth.

3.4. Spectrum of activity

Most products demonstrate satisfactory effectiveness against bacteria and enveloped viruses, such as HIV, hepatitis B and C, herpes, and influenza. In contrast, their activity against non-enveloped viruses (such as poliovirus, hepatitis A and E, and papillomavirus), mycobacteria (notably *Mycobacterium tuberculosis*), molds, or spores can vary significantly depending on the product. Therefore, the choice of product will depend on the type of disinfection required and the objectives to be achieved (Table 1).

Table 1. Spectrum of activity of main antiseptics and disinfectants (Yemiş and Harmancı., 2020).

Families	Spectrum of activity							
	Gram+	Gram-	Mycobacteria	Yeasts	Molds	Non-enveloped viruses	Enveloped viruses	Spores
Alcohols	+	+	+	+/-	+/-	+/-	+	-
Aldéhydes	+	+	+	+	+	+	+	+
Quaternary ammonium compound	+	+/-	-	+	+	+/-	+	-
Biguanides	+	+	+/-	+	+/-	+/-	+	-
Chlorine and iodine halogens	+	+	+	+	+	+	+	+
Oxidizing disinfectants	+	+	+	+	+	+	+	+
Oxidizing antiseptics	+	+	-	+	+	+/-	+	-

+ Active product / +/- Inconstantly active product / - Inactive product

Note:

- **Aldehydes**: for disinfection use only
- **Iodine halogens**: for antisepsis use only

3.5. Modes of action

Antiseptics and disinfectants exert their effects by disrupting the cellular structures or vital functions of microorganisms (Table 2), with their effectiveness depending on the type of product, concentration, duration of exposure, and the targeted microorganism. Their modes of action include protein denaturation, where certain chemical agents such as alcohols, phenols, or aldehydes disrupt microbial proteins, leading to inactivation or death; disruption of cell membranes, caused by substances like quaternary ammonium salts or some topical antibiotics, resulting in loss of cellular contents and cell death; inhibition of enzymes and metabolic

functions**,** where agents such as iodophors or active chlorine block essential enzymes or metabolic pathways, preventing reproduction; oxidative action**,** in which compounds like peroxides, hydrogen peroxide (H_2O_2), or permanganate generate free radicals that destroy cellular components; and action on genetic material, where certain specific chemicals interact with DNA or RNA, causing mutations or strand breaks that halt microbial reproduction.

Table 2. Modes of action of disinfectants (Piffaretti, 2006).

Classes	Examples	Targets and mechanism of action
Alcohols	Ethanol, Isopropanol	Denaturation of cytoplasmic and membrane proteins, inhibition of nucleic acid and protein synthesis
Formaldehyde	Formaldehyde	Alteration of the cell wall, inhibition of nucleic acid and protein synthesis
Quaternary ammonium compounds	Benzalkonium	Binding to fatty acids and phosphate groups of the cell membrane, leakage of cellular components, and cell lysis
Biguanides	Chlorohexidine	Binding to fatty acids and phosphate groups of the cell membrane, leakage of cellular components, and coagulation of the cytosol
Chlorine and Iodine halogens	Sodium hypochlorite (Bleach, Dakin)	Destruction of membrane and chromosomal proteins (halogenation).
Oxidants	Hydrogen peroxide	Production of free radicals that interact with lipids, proteins, and DNA

3.6. Examples of application

Question 1:

Indicate whether the following statements are true or false. If a statement is false, provide a justification.

1. To disinfect a wound, one can use Betadine.
2. Bleach loses its activity with use.

3. Aldehydes have a bactericidal effect on Gram-positive bacteria and a bacteriostatic effect on Gram-negative bacteria.
4. Propanol is an antiseptic, but isopropanol is a disinfectant.

Question 2:

Compare antiseptics and disinfectants in terms of usage, target surfaces, and spectrum of activity. Provide examples for each.

4. Antibiotics

Antibiotics represent one of the most significant discoveries in modern medicine, revolutionizing the treatment of bacterial infections. Their use has saved millions of human lives and significantly reduced mortality from severe bacterial infections. However, this scientific advancement is not without its challenges. Indeed, the growing resistance of bacteria to antibiotics has become a major public health issue, threatening the effectiveness of these treatments. This chapter aims to explore the world of antibiotics: their classification, their mechanisms of action, as well as the risks associated with their misuse. It also addresses the issue of antimicrobial resistance; a complex phenomenon that could undermine some of the most commonly used treatments today. Through this section, we will seek to understand not only the importance of these medications but also the precautions and strategies needed to preserve their effectiveness against increasingly resistant bacteria.

4.1. Discovery of antibiotics

Penicillin, the first antibiotic used clinically, is produced by *Penicillium notatum*. Its discovery dates back to 1929, when Alexander Fleming accidentally observed the inhibition of the growth of *Staphylococcus aureus* by a mold of the *Penicillium* genus, following an unintended contamination on a Petri dish. He demonstrated that extracts from this fungus were bactericidal without being toxic to animal cells and named the active substance “penicillin.” However, because the molecule was unstable, it was initially difficult for him to establish the validity of his findings.

It was not until 1939, in the context of World War II, that Howard Florey and Ernst Boris Chain succeeded in purifying penicillin on a large scale and conducting successful clinical trials, enabling its therapeutic use.

Subsequently, other major antibiotics were discovered: in 1944, Selman Waksman identified streptomycin from *Streptomyces griseus*; in 1953, Edward Abraham and Guy Newton isolated cephalosporin C from *Cephalosporium acremonium*.

Since 1965, a new era has emerged with the development of semi-synthetic antibiotics, particularly β-lactams.

4.2. Definition and modes of action

The term “antibiotic” derives from the Greek *anti* (“against”) and *bios* (“life”) and refers to a substance capable of inhibiting the development of microorganisms. Antibiotics are molecules of natural or synthetic origin, active at very low concentrations (on the order of $\mu g/cm^3$), that exert a specific action on certain microorganisms. They may either inhibit bacterial growth

(bacteriostatic effect) or kill bacteria (bactericidal effect) without significantly affecting the host's eukaryotic cells. The same antibiotic may be bacteriostatic at low doses and bactericidal at higher doses.

The distinction between bacteriostatic and bactericidal activity is based on the *in vitro* comparison of the MIC (minimum inhibitory concentration) and the MBC (minimum bactericidal concentration). An antibiotic is considered bactericidal when its MBC is close to its MIC. In contrast, when the MBC is much higher than the MIC and this concentration cannot be reached at the site of infection *in vivo*, the antibiotic is considered bacteriostatic.

4.3.Classification of antibiotics

Antibiotics are numerous and can be distinguished according to different criteria:

4.3.1. According to their origin

Antibiotics can be classified according to their origin as natural, semi-synthetic, or fully synthetic (Figure 12).

- **Natural antibiotics** are molecules produced by microorganisms. Among them, certain fungi, such as *Penicillium* and *Cephalosporium* species, produce penicillin, griseofulvin, and cephalosporins, while some bacteria, notably of the genus *Streptomyces*, synthesize streptomycin, chloramphenicol, kanamycin, nystatin, and other antibiotics. Gentamicin is produced by *Micromonospora* species, and bacitracin and polymyxins by *Bacillus* species.
- **Semi-synthetic antibiotics** are obtained by chemically modifying a natural molecule with the addition of a chemical group.
- **Synthetic antibiotics** are entirely produced through chemical synthesis, such as sulfonamides and nalidixic acids**.**

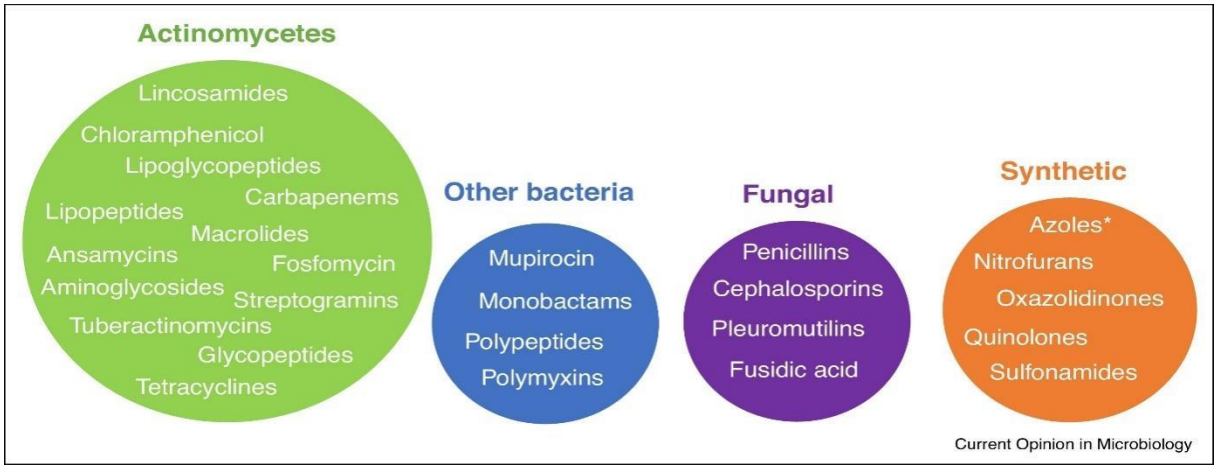


Figure 12. Most clinically relevant classes of antibiotic are derived from natural products (Hutchings et al., 2019).

4.3.2. According to their spectrum of activity

The spectrum of activity (or action) of an antibiotic refers to all microbial species for which the majority of strains are susceptible to that antibiotic. When it applies to only a limited number of species, the spectrum is described as "narrow," whereas an antibiotic effective against many bacteria is said to have a "broad spectrum."

4.3.3. According to their chemical relatedness

Antibiotics can be classified according to their chemical structure. This means that molecules sharing a common chemical core (Figure 13) (or a similar basic structure) are grouped into the same antibiotic family.

This classification is not merely theoretical; it has practical significance, because antibiotics within the same family generally have:

- **A similar mechanism of action**: for example, they may inhibit bacterial cell wall synthesis, protein synthesis, or disrupt the cell membrane.
- **A comparable spectrum of activity**: bacteria that are sensitive or resistant to one antibiotic in a family are often similarly affected by other members of the same family.

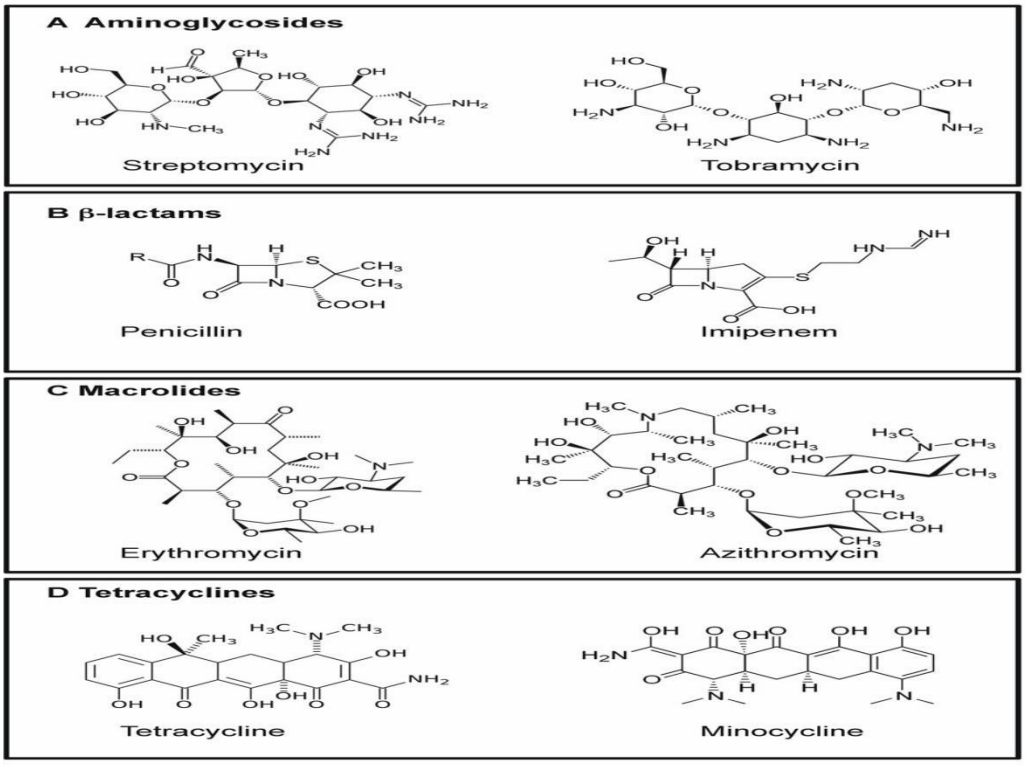


Figure 13. Examples of antibiotics classified according to their chemical relatedness (Romero et al., 2011).

4.3.4. According to their site of action

Antibiotics specifically target bacteria by inhibiting essential steps in their development, such as the synthesis of the cell wall, proteins, or nucleic acids, or by blocking metabolic pathways through the inhibition of key enzymes (Figure 14).

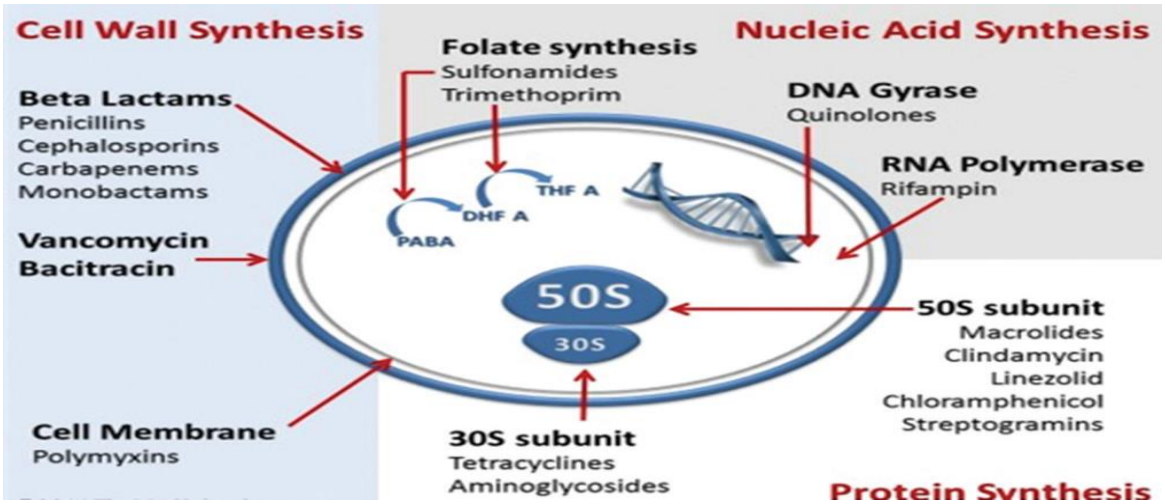


Figure 14. Site of action of antibiotics (Kapoor et al., 2017).

4.4.Mechanism of action of antibiotics

4.4.1. Antibiotics acting on peptidoglycan biosynthesis

Peptidoglycan is the most important component of the bacterial cell wall. N-acetylmuramic acid (NAM) alternates with N-acetylglucosamine (NAG), and the two are linked by chains of amino acids to form the polymer known as peptidoglycan. The synthesis of peptidoglycan involves several steps, ultimately leading to the formation of the bacterial cell wall. A peptidoglycan precursor is created by combining N-acetylglucosamine (NAG) with N-acetylmuramic acid (NAM). This precursor is then transported across the membrane and binds to cell wall receptors located in the periplasm. The peptidoglycan precursors subsequently undergo extensive cross-linking. Transpeptidase and carboxypeptidase enzymes are primarily responsible for this cross-linking process.

Several classes of antibiotics target these enzymes involved in cell wall synthesis (Figure 15), such as β-lactams (penicillins and cephalosporins), glycopeptides, fosfomycin, and bacitracin, among others. These antibiotics are bactericidal and act only on bacteria in the active phase of multiplication.

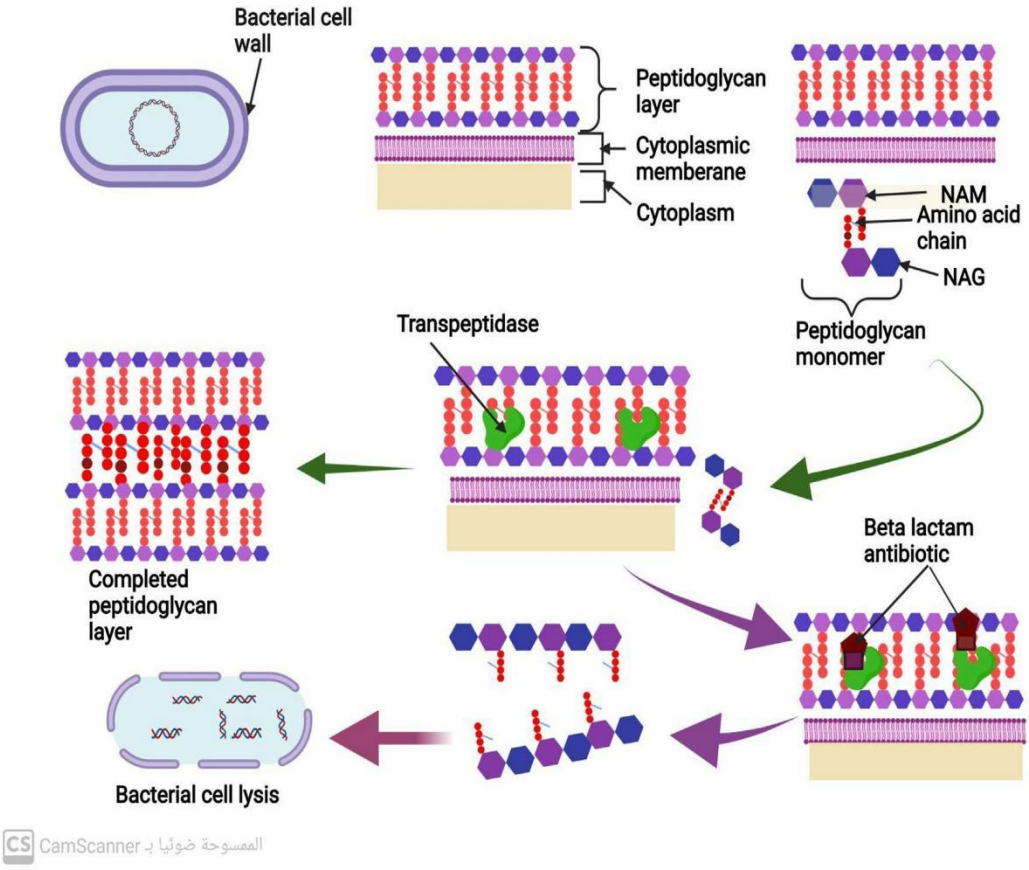


Figure 15. Cell wall synthesis and antibiotic inhibition mechanisms (Halawa et al., 2024).

4.4.2. Antibiotics inhibiting protein synthesis

Protein biosynthesis begins with the unwinding and separation of the DNA molecule at a region encoding the protein to be produced. Transcription, which corresponds to mRNA synthesis, uses only one DNA strand as a template. Once the mRNA molecule is synthesized, it detaches from the DNA and binds to a ribosome, composed of 50S and 30S subunits in bacteria. After these subunits assemble along the mRNA, translation begins with the formation of the polypeptide chain. The ribosome successively adds amino acids until it encounters the termination signal on the mRNA, at which point the complete polypeptide chain is released (Figure 16).

For antibiotics to inhibit protein synthesis, they specifically target the 30S or 50S ribosomal subunits. Several types of inhibitory antibiotics are distinguished:

- **50S subunit inhibitors**: these antibiotics either block the addition of a new amino acid to the polypeptide chain (phenicols) or prevent the transfer of the polypeptide chain from the A site to the P site on the ribosome (macrolides).

- **30S subunit inhibitors**: these antibiotics prevent or disrupt the binding of aminoacyl-tRNA to the ribosome (tetracyclines, aminoglycosides) and interfere with accurate genetic message reading. Some, such as streptomycin, cause misreading of the genetic code, leading to the incorporation of incorrect amino acids and the production of so-called “nonsense” proteins, which are genetically lethal to the cell.

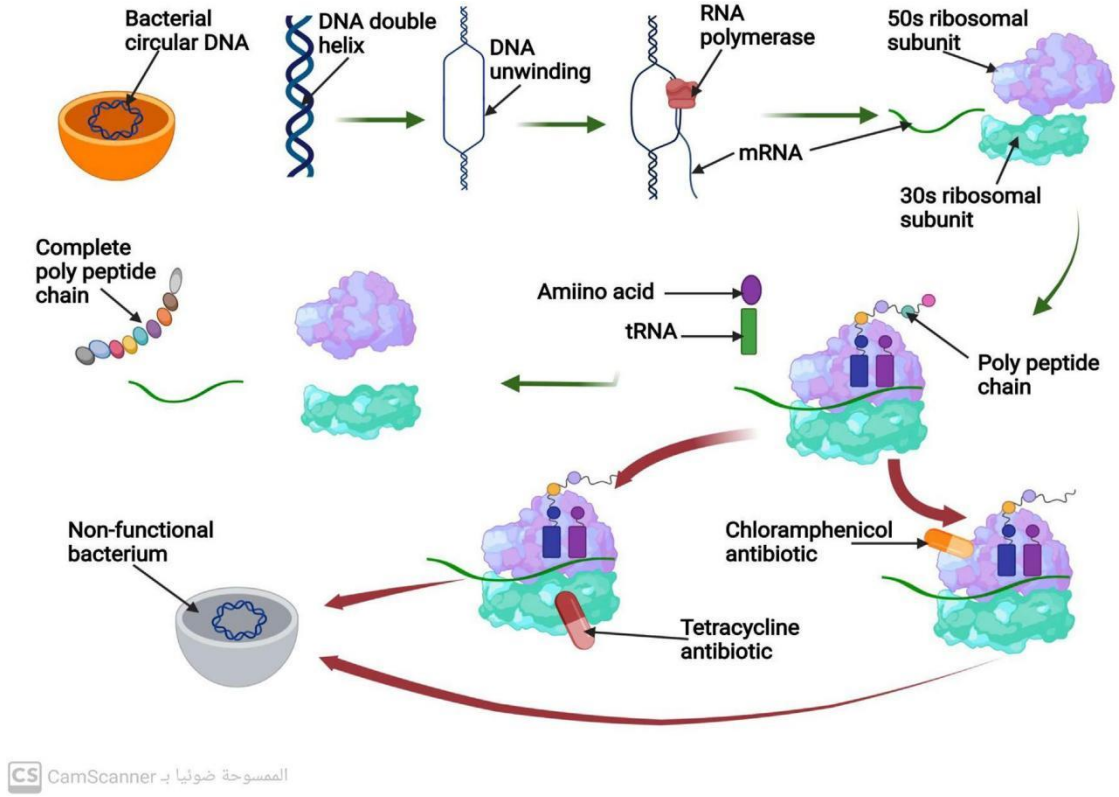


Figure 16. Protein biosynthesis and the mechanisms of antibiotic inhibition (Halawa et al., 2024).

4.4.3. Antibiotics inhibiting nucleic acid synthesis

Binary fission is the primary mode of bacterial cell division and first requires the duplication of circular DNA. This process begins with the separation of the two DNA strands by DNA helicase, followed by the synthesis of complementary strands by DNA polymerase. This enzymatic activity generates positive supercoils, which are corrected by DNA gyrase (topoisomerase II), a key enzyme also involved in transcription and the initiation of replication. After the formation of two interlinked DNA molecules, topoisomerase IV intervenes to separate them, allowing the formation of two daughter cells. Fluoroquinolones exert their action by inhibiting DNA gyrase and topoisomerase IV (Figure 17), thereby blocking bacterial DNA synthesis: by binding to the enzyme–DNA complex, they induce DNA breaks and lead to cell

death. They primarily target DNA gyrase in Gram-negative bacteria, whereas in Gram-positive bacteria their action mainly focuses on topoisomerase IV, disrupting the separation of DNA molecules.

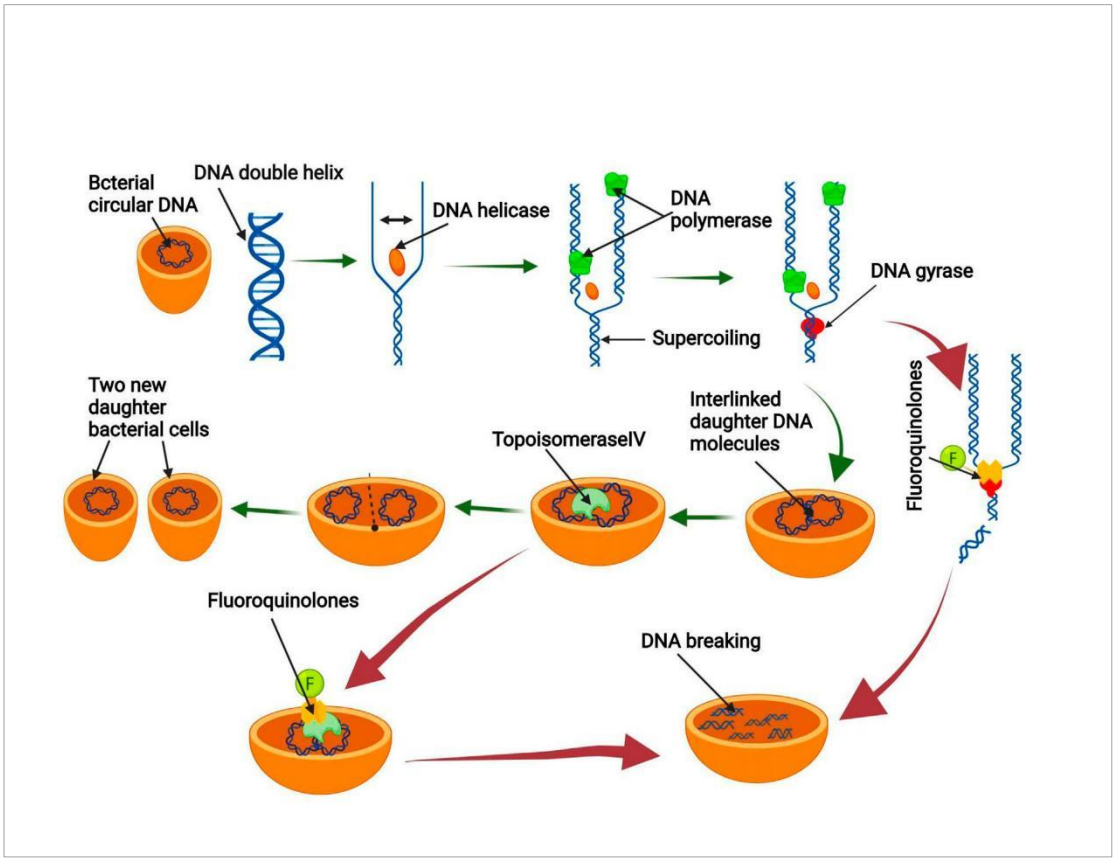


Figure 17. DNA replication process and the mode of action of antibiotics that inhibit this replication (Halawa et al., 2024).

4.4.4. Antibiotics acting by competitive inhibition of metabolic pathways

Antibiotics acting by competitive inhibition of metabolic pathways target enzymes essential for bacterial function, thereby preventing the synthesis of vital compounds and disrupting cellular metabolism. This mode of action inhibits bacterial growth and multiplication. More specifically, these antibiotics selectively inhibit key enzymes in folic acid metabolism: sulfonamides target dihydropteroate synthase, while trimethoprims act on dihydrofolate reductase, two distinct enzymes within the same metabolic pathway (Figure 18).

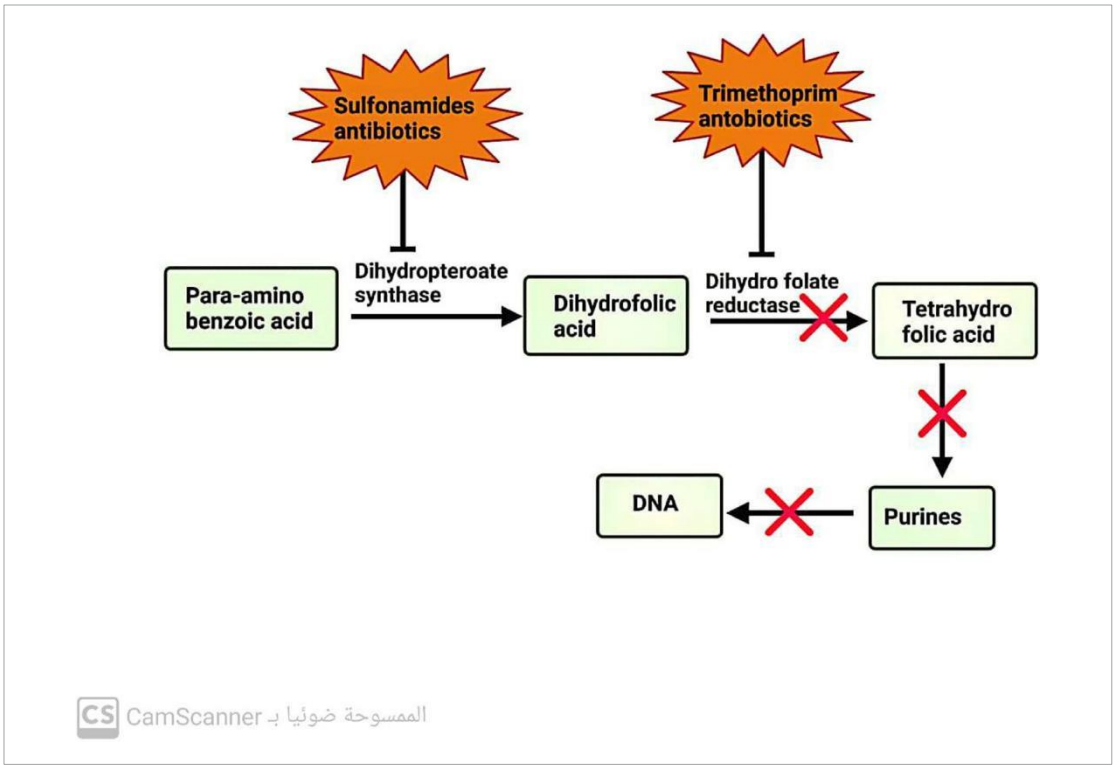


Figure 18. Mode of action of antibiotics that block folic acid metabolism (Halawa et al., 2024).

4.5.Types of resistance

Antibiotic resistance in bacteria can either be a natural characteristic of the organism, such as a specific cell wall that makes it intrinsically resistant, or an acquired resistance through mutation of its DNA or the incorporation of resistance-conferring DNA from another source.

- **Natural (intrinsic) resistance:** Some bacteria possess an innate resistance to an antibiotic. This may be due to the absence of a transport system allowing the antibiotic to enter, the absence of the antibiotic's molecular target, or, as in Gram-negative bacteria, the presence of an outer membrane that acts as a permeability barrier.
- **Acquired resistance:** To develop resistance to antibiotics, bacteria can either modify their existing genetic material or acquire new genes from another source, thereby gaining the ability to withstand treatment.

4.6. Mechanism of resistance

Bacterial resistance to antibiotics is today one of the main threats to global public health, compromising the effectiveness of treatments and the fight against infections. It results from the adaptation of bacteria to the pressures exerted by excessive or inappropriate use of

antibiotics. Bacteria employ three main strategies to defend against the effects of antibiotics:

4.6.1. Bacteria block the accumulation of antibiotics inside their cells

4.6.1.1. Restricting the penetration of drugs into bacterial cells

Gram-negative bacteria have channels called porins in their outer membrane which act as selective gateways, allowing only certain antibiotics, such as beta-lactams and quinolones, to enter the cell. A decrease in the number of these porins can thus reduce antibiotic entry, leading to increased resistance to these treatments (Figure 19).

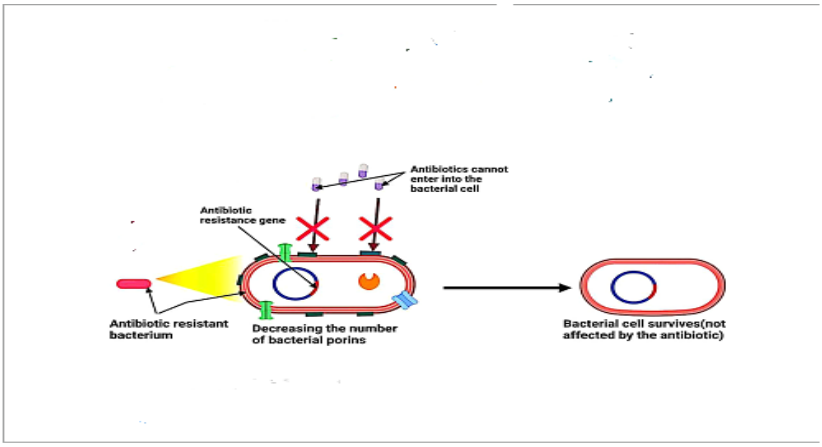


Figure 19. Mechanism of antibiotic resistance by decreasing antibiotic entry into the bacterial cell. (Halawa et al., 2024).

4.6.1.2. Acceleration of antibiotic expulsion from bacterial cells

Membrane proteins that expel antibiotics from the cell and thus maintain low intracellular concentrations are called efflux pumps. These mechanisms (Figure 20) pump antibiotics out at the same rate as they enter the cell, preventing them from reaching their target. Unlike porins located in the outer membrane, these pumps are found in the cytoplasmic membrane. All antibiotics, except polymyxin, can be affected by the activation of efflux systems.

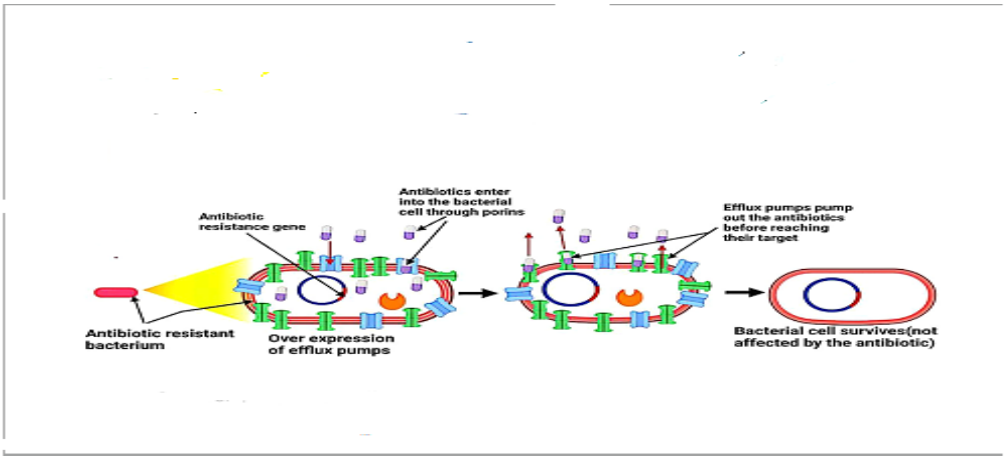


Figure 20. Mechanism of antibiotic resistance by increasing antibiotic exit from the bacterial cell (Halawa et al., 2024).

4.6.2. Bacteria alter the molecule targeted by antibiotics

Bacteria can become resistant to antibiotics through natural variations or acquired modifications of their target sites, preventing the drug from binding effectively. These target alterations often result from spontaneous mutations in a bacterial gene located on the chromosome. Since the interaction between an antibiotic and its target is usually highly specific, even minor changes in the target molecule can significantly reduce the effectiveness of antibiotic binding

4.6.2.1. Alteration of penicillin-binding proteins (PBP)

In Gram-positive bacteria, modification of penicillin-binding proteins **(**PBPs**)** is a common resistance mechanism. Mutations affecting PBPs reduce their affinity for β-lactam antibiotics, thereby decreasing their effectiveness (Figure 21).

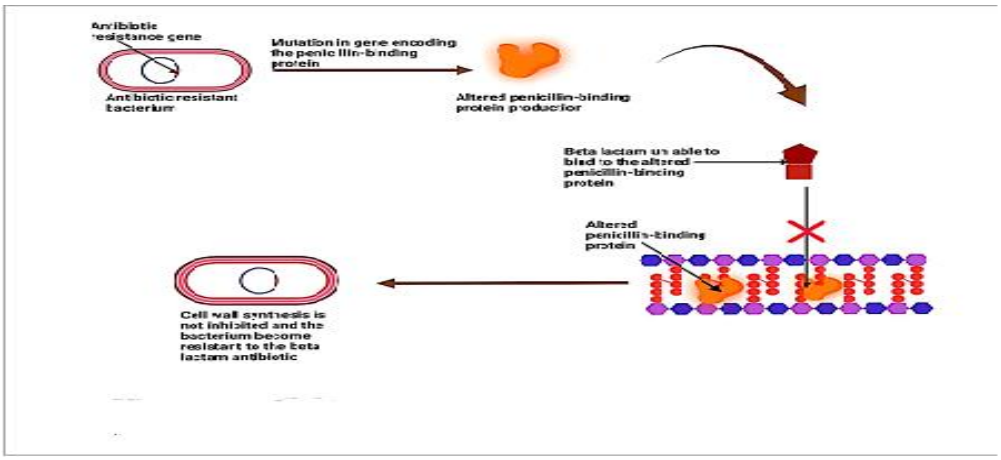


Figure 21. Mechanism of antibiotic resistance by alteration in penicillin-binding protein (Halawa et al., 2024).

4.6.2.2.Alteration in the 30S subunit or 50S subunit

Bacteria can develop resistance to antibiotics that disrupt protein synthesis by modifying their 30S or 50S ribosomal subunits (Figure 22). This resistance mechanism affects antibiotics such as aminoglycosides, tetracycline, macrolides, chloramphenicol, lincosamides, and streptogramin.

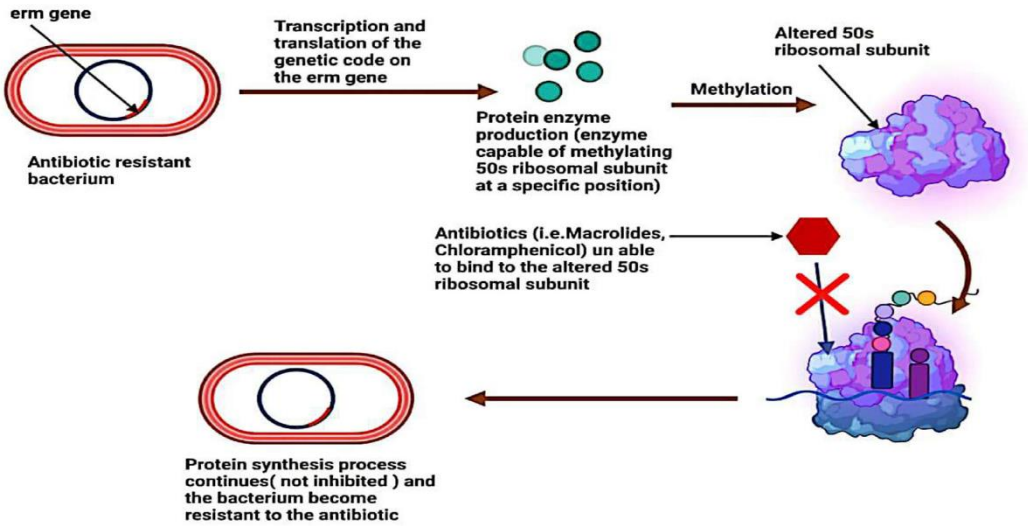


Figure 22. Mechanism of antibiotic resistance by alteration in 50 s ribosomal subunit (Halawa et al., 2024).

4.6.2.3. Modifications in DNA gyrase and topoisomerase enzymes

DNA replication depends on the enzymes DNA gyrase and topoisomerase. Quinolones act by specifically targeting these two enzymes, and any modification in their structure can lead to the development of bacterial resistance to these antibiotics (Figure 23).

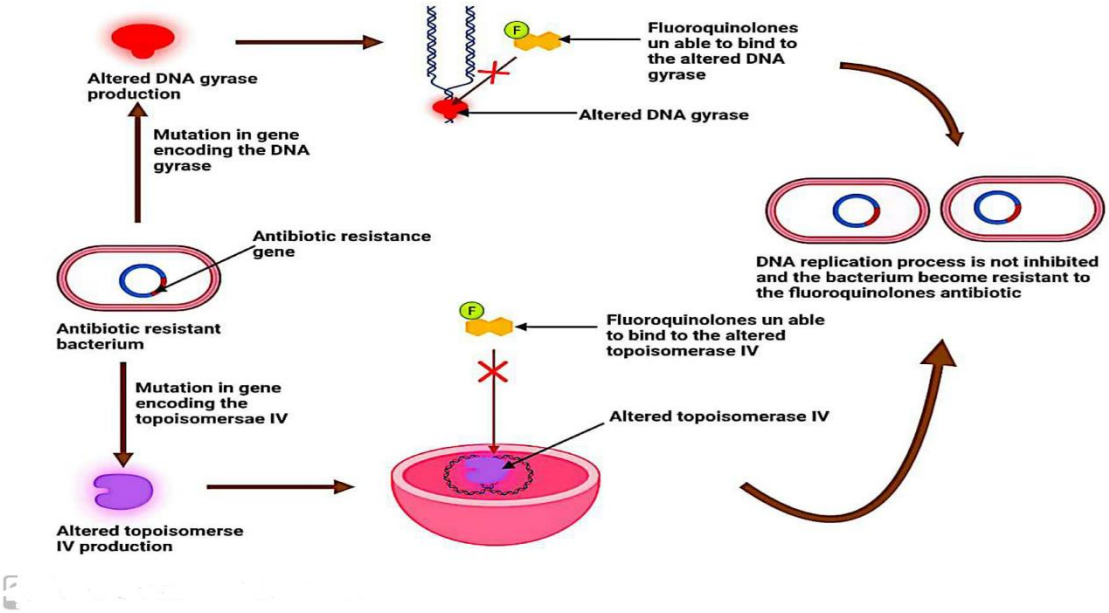


Figure 23. Mechanism of antibiotic resistance by alteration in DNA gyrase and topoisomerase IV (Halawa et al., 2024).

4.6.2.4. Altered cell wall precursors

Peptidoglycan precursors contain a dipeptide residue, D-alanyl-D-alanine, which is essential for cell wall formation. In Gram-positive bacteria, glycopeptides such as vancomycin or teicoplanin normally inhibit cell wall synthesis by binding to this residue. However, the replacement of D-alanyl-D-alanine with D-alanyl-lactate prevents these antibiotics from binding and forming the necessary cross-links, leading to the development of bacterial resistance.

4.6.2.5.Ribosomal protection mechanisms

Ribosomal protection mechanisms confer resistance to tetracyclines by preventing these antibiotics from binding to ribosomes, allowing bacterial protein synthesis to continue despite the presence of the drug.

4.6.2.6. RNA polymerase mutations

RNA polymerase mutations refer to genetic changes that affect the enzyme responsible for transcribing DNA into RNA. These mutations can alter the enzyme's structure, preventing antibiotics such as rifamycins from binding effectively. As a result, the bacteria can continue RNA synthesis and survive despite the presence of the antibiotic.

4.6.3. Bacteria neutralize the antibiotic through enzymatic activity

Three main enzymes (Figure 24) are responsible for antibiotic inactivation: β-lactamases, aminoglycoside-modifying enzymes (AGE's), and chloramphenicol acetyltransferases (AACs).

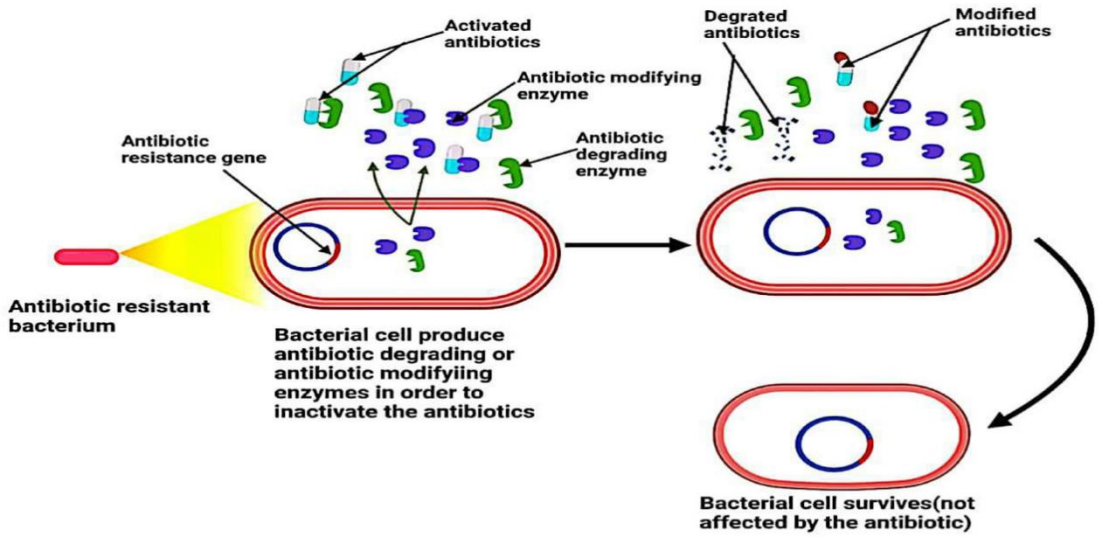


Figure 24. Mechanism of antibiotic resistance by inactivation of the antibiotic (Halawa et al., 2024).

4.6.3.1. Beta-lactamases

β-lactamases are bacterial enzymes that hydrolyze β-lactam antibiotics containing ester and amide bonds such as penicillins, cephalosporins, monobactams, and carbapenems thereby conferring resistance; around 300 types have been identified to date.

4.6.3.2. Aminoglycoside modifying enzymes

Aminoglycoside-modifying enzymes (AMEs) prevent aminoglycosides from binding to their ribosomal target. Found in *E. faecalis*, *S. aureus*, and *S. pneumoniae*, they also contribute to resistance to fluoroquinolones, thereby reducing the effectiveness of these treatments.

4.6.3.3. Chloramphenicol-acetyl-transferases

Chloramphenicol-acetyl-transferases are enzymes that modify the antibiotic chloramphenicol by acetylating its hydroxyl groups. This generates an altered form of the antibiotic, which is unable to bind to its ribosomal target. As a result, bacteria producing this enzyme become resistant to chloramphenicol, making this antibiotic ineffective. Some Gram-positive and Gram-negative bacteria, as well as certain strains of *Haemophilus influenzae*, possess this enzyme, which prevents the correct binding of chloramphenicol to the 50S ribosomal subunit.

4.7. Association of antibiotics

The association of antibiotics consists of administering two or more antibiotics simultaneously in order to improve the effectiveness of treatment against an infection. This strategy is used in several situations, particularly to:

- Broaden the spectrum of action when the responsible microorganism is not yet

identified,

- Achieve a synergistic effect where antibiotics work better together,
- Prevent the emergence of bacterial resistance as in tuberculosis,
- Treat infections involving multiple types of bacteria.

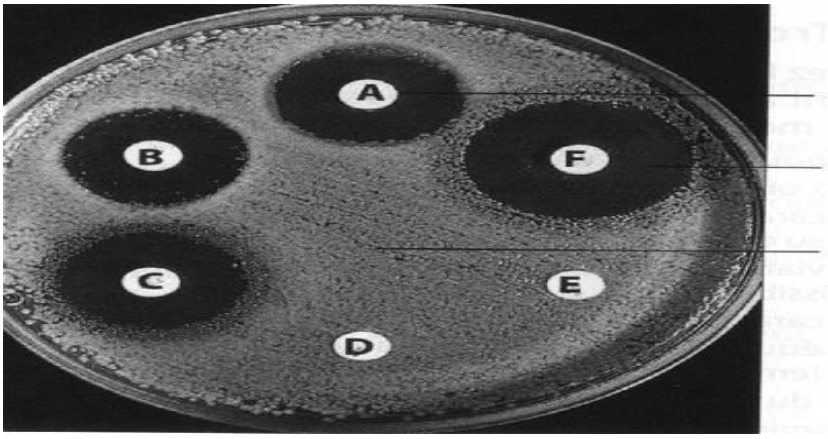
➢ **Examples of associations**

- Amoxicillin + clavulanic acid
- Cephalosporin + aminoglycoside
- Trimethoprim + sulfamethoxazole

4.8. Examples of application

Question 1:

After 18 hours of culture, the image of the antibiogram shown in the figure below is obtained.



Complete the labels on the image.

Based on the diagram, complete with true or false.

To combat this bacterium:

a. Antibiotic F will be the most effective

b. Antibiotics A, B, and C will be the most effective

c. Antibiotics A, B, and C will have the same effectiveness

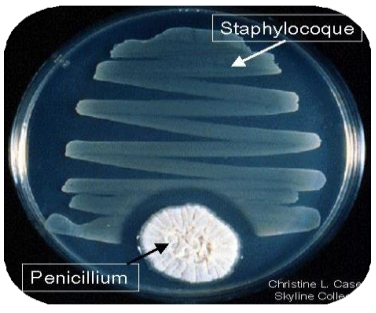
d. Antibiotic E will need to be used at a high concentration to be effective

Question 2:

1.1.In 1928, a scientist observed a Petri dish of *Staphylococcus* similar to this one.

1.2.What did he observe on this dish?

1.3. Who was he?



2. Define the word “antibiotic.”
3. Give an example of an antibiotic-producing bacterium and an example of an antibiotic-producing fungus and bacterium

5. Antifungals

Antifungal agents constitute an essential class of medications used in the management of infections caused by fungi, known as mycoses. Long overlooked compared to bacterial infections, these conditions now represent a major public health concern, particularly among immunocompromised patients. The development of antifungal drugs, from the discovery of molecules such as Nystatin and Amphotericin B to more recent treatments like Fluconazole, has significantly improved the prognosis of these infections. This chapter aims to present the different classes of antifungal agents and their mechanisms of action.

5.1. History of antifungals

The history of the discovery of antifungals begins in the early 20th century with the identification of the first substances capable of inhibiting the growth of fungi. In the 1950s, nystatin, the first effective and safe antifungal, was discovered by two American researchers, marking a key milestone in the treatment of fungal infections. Subsequently, amphotericin B was introduced in the 1960s, becoming a reference treatment for severe infections. The following decades saw the emergence of new classes, such as azoles in the 1970s and 1980s, which offered better tolerance and simpler administration. Today, research continues to evolve to address the challenges posed by fungal resistance and to improve treatment efficacy.

5.2. Definition and modes of action

Antifungals are drugs or chemical substances used to prevent or treat infections caused by fungi. They can be topical, applied directly to the skin, mucous membranes, or nails to treat superficial infections, or systemic, administered orally or by injection to reach deep and internal infections. Their action relies on mechanisms targeting structures or functions specific to fungal cells, which limits effects on human cells. Depending on their effect on fungi, antifungals are classified as fungicidal, which directly kill fungal cells (such as amphotericin B and echinocandins), and fungistatic, which inhibit the growth and reproduction of fungi (such as azoles and flucytosine).

5.3. Classification of antifungals

Antifungals are divided into five main classes based on their chemical structure and mode of action.

- **Polyenes**, such as amphotericin B and nystatin, are generally fungicidal and act by binding to ergosterol in the fungal membrane, causing leakage of cellular contents.

- **Azoles**, such as fluconazole, itraconazole, or ketoconazole, are primarily fungistatic and inhibit ergosterol synthesis, disrupting the fungal membrane.
- **Allylamines**, such as terbinafine and naftifine, are also fungistatic or sometimes fungicidal and block an enzyme essential for ergosterol production, making them effective against skin and nail infections.
- **Echinocandins**, such as caspofungin, micafungin, and anidulafungin, are fungicidal and inhibit β-glucan synthesis in the cell wall, mainly used for severe systemic infections.
- **Flucytosine** acts in a fungistatic manner by blocking fungal DNA and RNA synthesis and is often combined with amphotericin B for severe infections.

5.4. Mechanisms of action of antifungals

Antifungals act by targeting structures or processes specific to fungal cells. The targets of all antifungals used in the clinic are summarized in Figure 25.

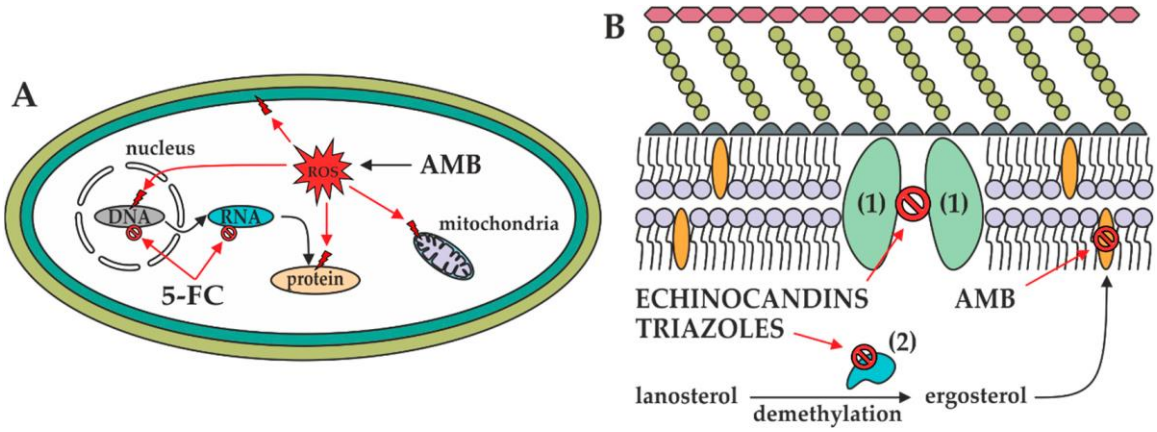


Figure 25. Mechanisms of actions of antifungals in the fungal cell.

(Houšť et al., 2020).

5.4.1. Disruption of the fungal cell membrane

This mechanism involves targeting and directly damaging the plasma membrane of fungal cells, thereby disrupting their integrity and leading to cell death. The fungal membrane contains a key molecule called ergosterol, which plays a role similar to cholesterol in human cells by maintaining membrane fluidity and stability. Antifungals that use this mechanism, such as

polyenes (Figure 26) (notably amphotericin B), bind specifically to ergosterol and form pores or channels in the membrane, causing leakage of essential cellular components such as ions, nutrients, and nucleic acids, which leads to cell destruction. It is also important to note that amphotericin B can induce the accumulation of reactive oxygen species (ROS), resulting in damage to DNA, proteins, mitochondria, and the membrane.

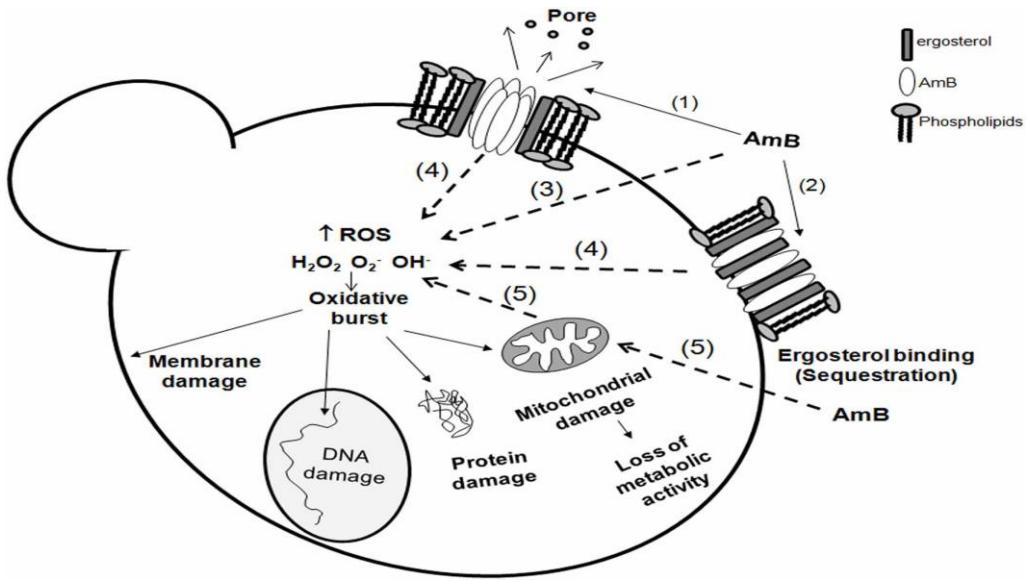


Figure 26. Amphotericin B action mechanisms on fungal cells. (Mesa-Arango et al., 2012).

5.4.2. Inhibition of ergosterol synthesis

Ergosterol is an essential component of the plasma membrane of fungi, playing a role similar to cholesterol in human cells by maintaining membrane fluidity, integrity, and function. Some antifungals act by blocking ergosterol production (Figure 27), leading to the formation of defective, unstable, and permeable fungal membranes. Among these antifungals:

- **Azoles** (fluconazole, itraconazole, ketoconazole) inhibit the enzyme 14α-demethylase, preventing the conversion of lanosterol to ergosterol.
- **Allylamines** (terbinafine, naftifine) block the enzyme squalene epoxidase, leading to the accumulation of toxic squalene and a decrease in ergosterol.

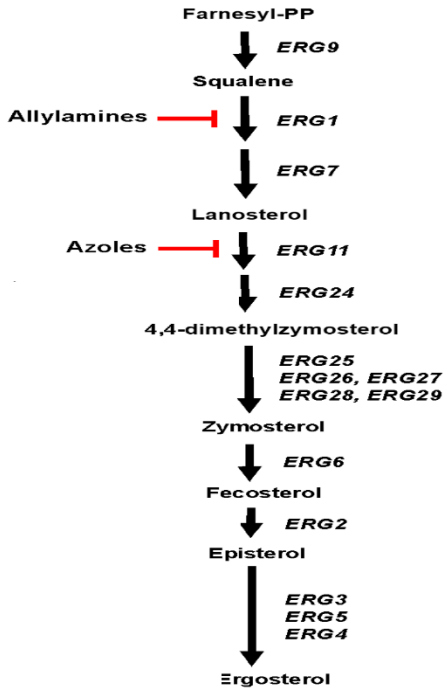


Figure 27. Azoles and alylamines actions mechanisms on ergosterol synthesis. (Eliaš et al., 2024).

5.4.3. Inhibition of fungal cell wall synthesis

The cell wall of fungi is an essential structure that protects the cell against environmental stresses and maintains its shape. It is mainly composed of polysaccharides, including β-glucan, chitin, and mannoproteins. Unlike human cells, fungal cells rely heavily on this wall for their survival, making it an ideal target for antifungals. Some antifungals, such as echinocandins (caspofungin, micafungin, anidulafungin), specifically inhibit β-glucan synthesis, a crucial component of the cell wall. By blocking this synthesis, the wall becomes fragile and unstable, leading to cell rupture and fungal death, giving this mechanism a fungicidal effect (Figure 28).

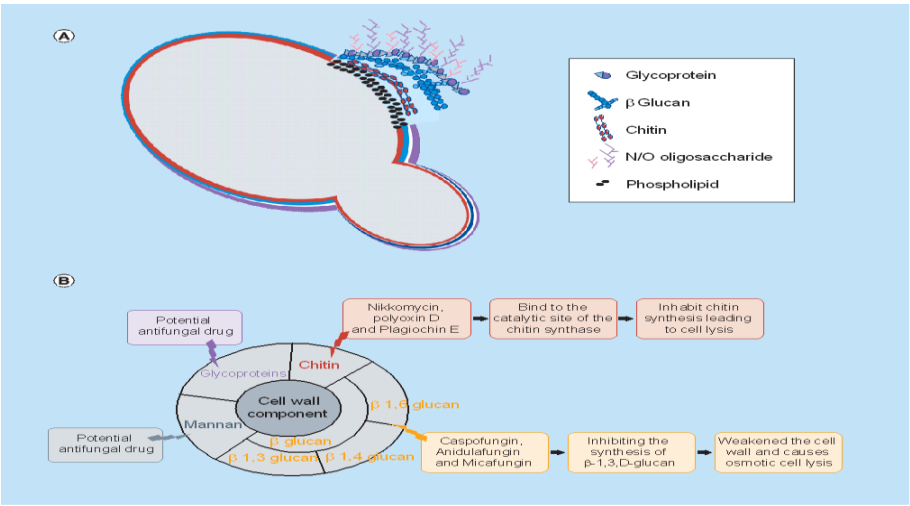


Figure 28. Structure of the fungal cell wall and overview of compounds that target components (Hasim and Coleman 2019).

5.4.4. Inhibition of nucleic acid or protein synthesis

This mechanism involves disrupting essential processes in the fungal cell, particularly the synthesis of DNA, RNA, or proteins, which are necessary for fungal growth and reproduction. Some antifungals, such as flucytosine (5-FC), enter the fungal cell through specific transporters and are then converted into active metabolites. These metabolites interfere with DNA and RNA synthesis, thereby blocking replication and protein production (Figure 29).

As a result, the fungal cell can no longer multiply or maintain its vital functions, leading to a growth arrest (fungistatic effect), although this effect may become fungicidal under certain conditions or when combined with other antifungal agents.

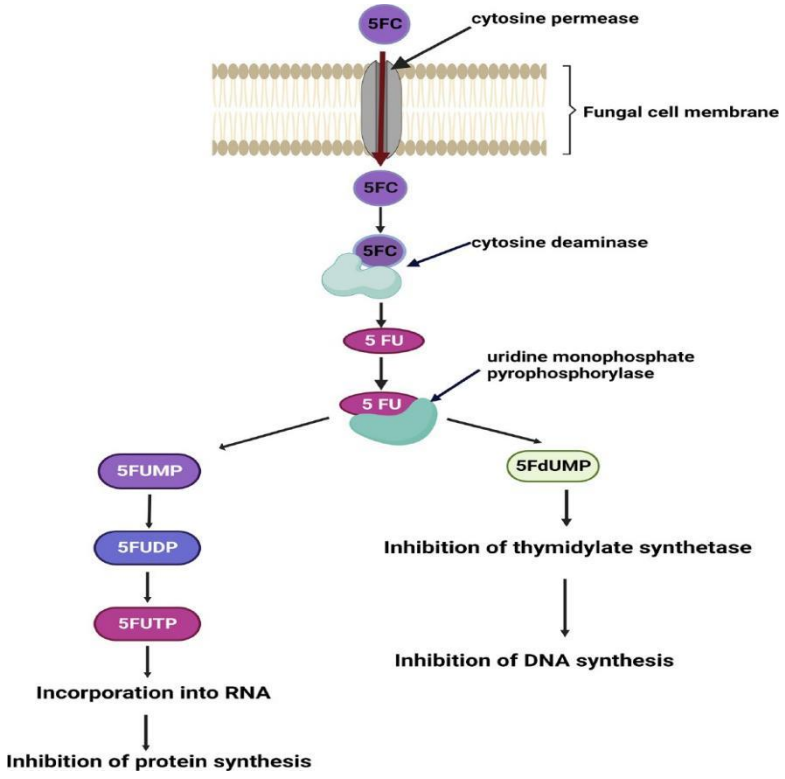


Figure 29. Diagram shows the mode of action of 5FC (Sigera and Denning, 2023).

5.5. Resistance to antifungal agents

Resistance to antifungal agents has become an increasing concern in the management of fungal infections. It occurs when fungi develop the ability to survive exposure to antifungal drugs that would normally inhibit their growth or kill them. This resistance can arise through various mechanisms (Figure 30), including genetic mutations and adaptive cellular responses. For instance, fungal cells may overproduce the target enzyme, preventing complete inhibition of the biochemical pathway, or alter the drug target, reducing or preventing drug binding.

Resistance can also result from the activation of efflux pumps that expel the drug from the cell, or from reduced drug entry at the level of the cell membrane or cell wall. Additionally, some fungi develop bypass pathways that compensate for the inhibited function, while others may inhibit enzymes responsible for converting inactive drugs into their active forms. In certain cases, fungal cells can even secrete enzymes into the extracellular environment that degrade the antifungal agent, further contributing to treatment failure.

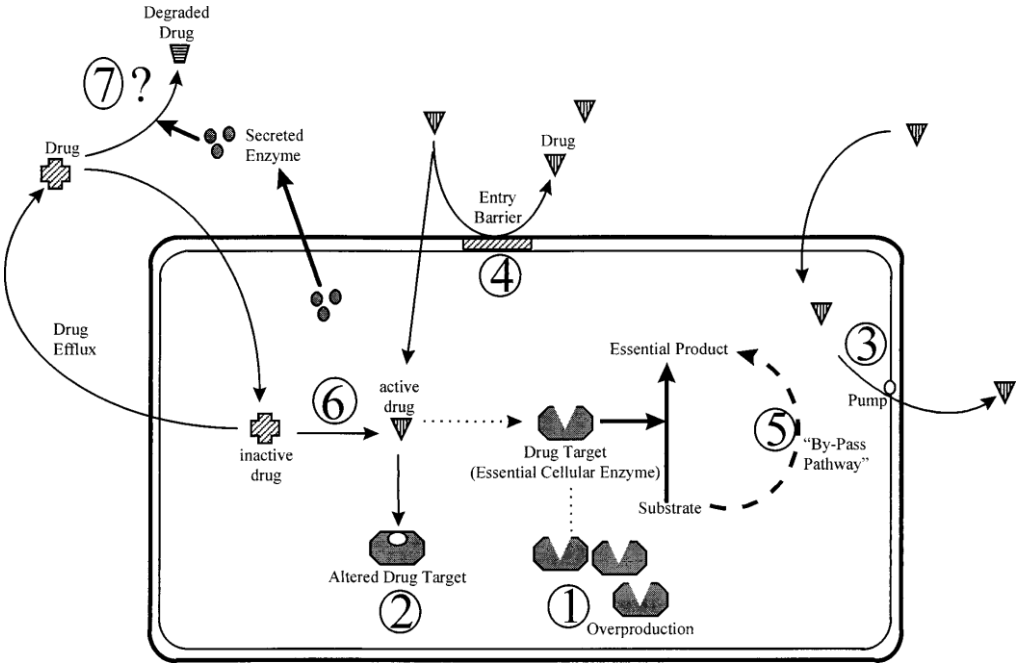


Figure 30. Mechanisms by which microbial cells might develop resistance. (Ghannoum and Rice, 1999).

1, The target enzyme is overproduced, so that the drug does not inhibit the biochemical reaction completely. 2, The drug target is altered so that the drug cannot bind to the target. 3, The drug is pumped out by an efflux pump. 4, The entry of the drug is prevented at the cell membrane/cell wall level. 5, The cell has a bypass pathway that compensates for the loss-of-function inhibition due to the drug activity. 6, Some fungal "enzymes" that convert an inactive drug to its active form are inhibited. 7, The cell secretes some enzymes to the extracellular medium, which degrade the drug.

4.6. Examples of application

- Give the definition of an antifungal and provide an example.
- What type of microorganisms do antifungals target? Give an example of a drug.

6. Antivirals

This chapter is devoted to antivirals; a class of drugs used to prevent and treat viral infections. Unlike antibiotics, which act against bacteria, antivirals specifically target the different stages of the viral replication cycle. We will discuss the mechanisms of action of antivirals as well as the obstacles of antiviral chemotherapy.

6.1. History of antivirals

The history of antivirals is relatively recent compared to that of antibiotics, due to the particularities of viruses, which use the host cell machinery to replicate. For a long time, the fight against viral infections relied primarily on prevention through vaccination.

A decisive breakthrough occurred in the 1960s with the discovery of idoxuridine, the first antiviral used in human medicine, marking the beginning of antiviral chemotherapy. Subsequently, the development of more selective molecules, such as acyclovir in the 1970s, improved therapeutic efficacy while reducing toxicity.

The Human Immuno deficiency Virus (HIV) epidemic in the 1980s represented a major turning point, stimulating research and leading to the emergence of combination antiretroviral therapies in the 1990s. Since the 2000s, advances in molecular biology have enabled the development of direct-acting antivirals that are more specific and effective.

Thus, antivirals now occupy an essential role in the management of viral infections, complementing vaccination strategies.

6.3. Definition

Antivirals are drugs designed to prevent or treat viral infections by specifically targeting the virus and its replication mechanisms. They do not act directly on the target cells themselves but on viral processes, while minimizing, as much as possible, effects on the host’s cells.

6.4. Mechanisms of action of antivirals

Available antiviral agents primarily target different stages of the viral life cycle (Figure 31). The stages targeted in the viral cycle include: virus attachment to the host cell, uncoating, viral mRNA synthesis, mRNA translation, viral RNA and DNA replication, maturation of new viral proteins, budding, release of newly synthesized viruses, and the presence of free virus in body fluids.

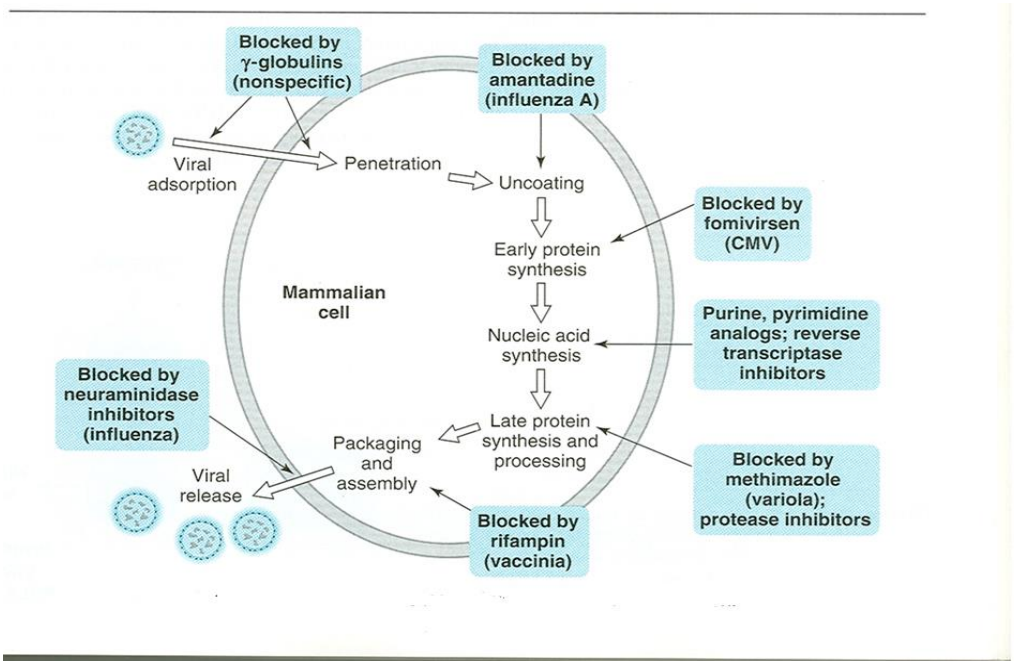


Figure 31. Possible general mechanism of action of antivirals (Bule et al., 2019).

6.4.1. Inhibition of viral attachment

It is the interaction between the virus and the host cell that allows the virus to attach to the cell surface (Figure 32).

This interaction involves receptors present:

- on the surface of the host cell, generally proteins or membrane glycoproteins,
- as well as viral proteins located on the envelope or capsid of the virus.

Each virus recognizes a specific receptor. For example, HIV uses the CD4 receptor as well as the coreceptors CCR5 or CXCR4 on T lymphocytes, whereas SARS-CoV-2 binds to the ACE2 receptor on respiratory cells, and the influenza virus binds to sialic acid. On the viral side, specific proteins such as gp120 (HIV), the Spike protein (SARS-CoV-2), or hemagglutinin (influenza) ensure this recognition.

Antiviral drugs can act by blocking these cellular receptors, inhibiting viral proteins, or preventing membrane fusion, thereby preventing the virus from entering the cell and initiating infection.

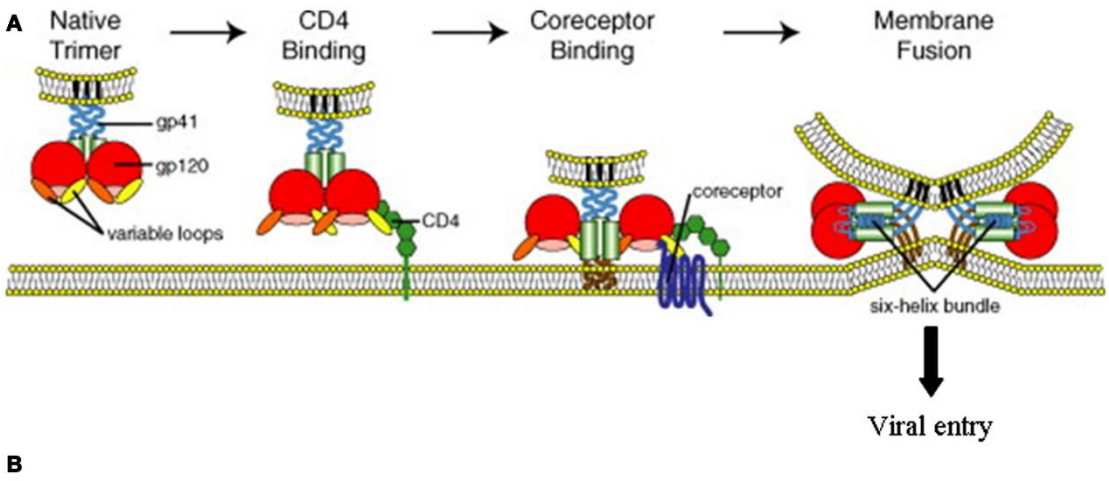


Figure 32. HIV attachment and entry mechanism (Connell and Lortat-Jacob,. 2013).

6.4.2. Inhibition of viral entry

Viruses must cross the host cell plasma membrane to initiate infection. To do so, they use different mechanisms (Figure 30) depending on their structure (enveloped or non-enveloped). Antiviral drugs can block viral entry by:

- Blocking the cellular receptors required for virus attachment,
- Inhibiting viral proteins responsible for fusion or DNA/RNA injection,
- Preventing endocytosis or membrane fusion.

Thus, inhibition of viral entry prevents the virus from crossing the plasma membrane and stops the infection.

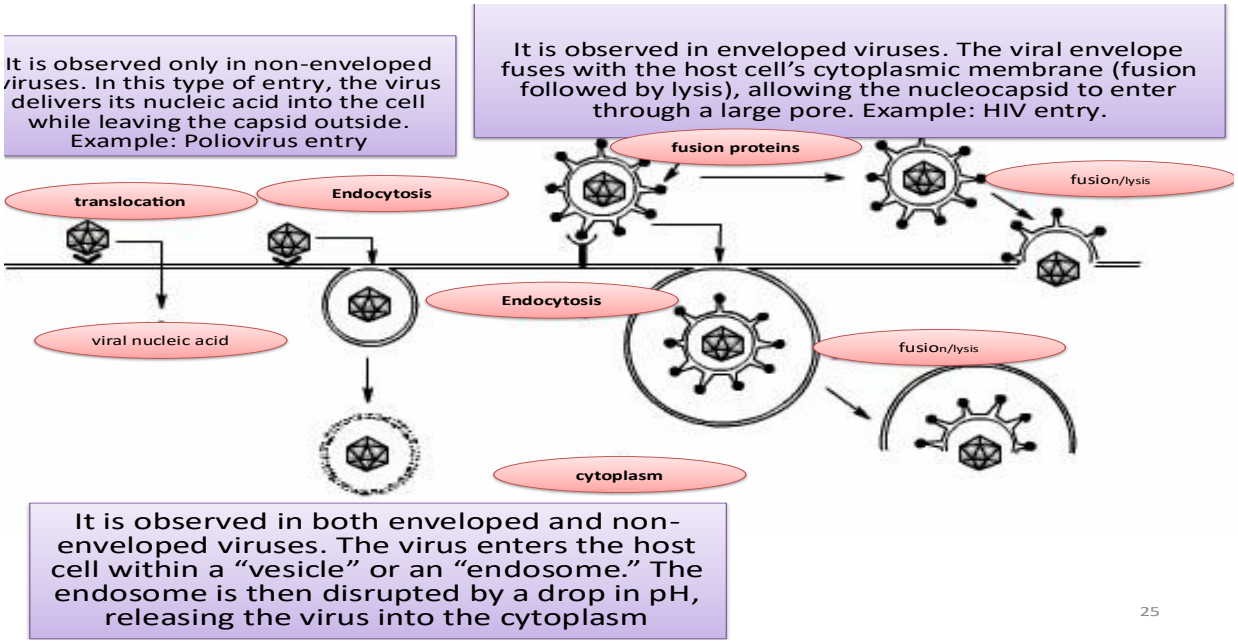


Figure 33. Mechanism of viral entry

6.4.3. Inhibition of uncoating

The inhibition of uncoating is an antiviral mechanism that prevents the virus from releasing its nucleic acid (DNA or RNA) inside the host cell after entry.

After the virus crosses the plasma membrane or is internalized into an endosome, its capsid must disassemble so that the viral genetic material becomes accessible to the cell and replication can begin.

Antivirals acting at this stage block the function of viral proteins or ion channels necessary for uncoating.

6.4.4. Inhibition of viral replication

The inhibition of replication is a mechanism by which antivirals prevent the virus from copying its genome (DNA or RNA) inside the host cell. Without replication, the virus cannot produce new virions, and the infection is stopped.

This mechanism works in several ways:

- **Nucleoside or nucleotide analogues**: These are molecules resembling natural nucleotides, which are incorporated into viral DNA or RNA during replication, causing synthesis to stop.
- **Viral polymerase inhibitors**: Directly block the enzyme responsible for synthesizing viral DNA or RNA.
- **Reverse transcriptase inhibitors** (for retroviruses): Prevent the conversion of viral RNA into DNA, a step necessary for integration into the host cell genome.

6.4.5. Integrase inhibitors

Integrase allows the virus to integrate its viral DNA into the host cell's DNA after the virus has been reverse-transcribed from its RNA. This step is crucial, as integration enables the virus to use the host cell's machinery to produce new viruses.

Integrase inhibitors block the activity of this enzyme, preventing the integration of viral DNA into the host cell genome. Without integration, the virus cannot replicate efficiently, thereby limiting the production of new virions.

6.4.6. Inhibition of viral maturation and assembly

The inhibition of maturation and assembly is a mechanism by which antivirals prevent the virus from forming functional viral particles after replication. Even if the viral genome has been copied and viral proteins have been synthesized, the virus cannot produce new infectious virions if assembly and maturation are blocked.

This mechanism works in two main ways:

- **Blocking viral proteases**: These enzymes cleave viral polyproteins into functional proteins, a step essential for the formation of mature virions.
- **Inhibition of viral component assembly**: Prevents the packaging of the viral genome and proteins into new particles.

6.4.7. Inhibition of viral release

The inhibition of viral release is a mechanism by which antivirals prevent newly formed viruses from leaving the host cell to infect other cells. Even if the virions are properly assembled and mature, their exit is blocked, thereby limiting the spread of infection.

This mechanism works mainly in two ways:

- **Blocking viral enzymes**: Some enzymes, such as neuraminidase in influenza viruses, are necessary to detach virions from the cell membrane. Antivirals that inhibit these enzymes prevent the release of viral particles.
- **Altering the cell membrane**: Some antivirals disrupt the budding process of virions from the plasma membrane.

6.5. Mechanisms of antiviral resistance

Viruses can develop resistance to antivirals, thereby reducing the effectiveness of treatments. This resistance generally results from changes in the viral genome that alter the drug targets or the viral processes themselves.

The main mechanisms include:

- **Mutations in viral target proteins**: Antivirals often act on specific viral enzymes or proteins (such as polymerase, protease, integrase, or neuraminidase). Mutations can change the structure of these proteins, preventing the drug from binding effectively.
- **Modification of binding sites or receptors**: Viruses can alter their envelope proteins or binding sites, reducing the affinity of antivirals that block entry or fusion.
- **Escape from enzymatic inhibition mechanisms**: Some viruses develop alternative enzymes or resistant variants that bypass the antiviral blockade, such as resistant polymerases or proteases.
- **Progressive accumulation of mutations (multimutant resistance)**: Prolonged exposure to antivirals favors the selection of viruses with multiple mutations, rendering certain treatments ineffective.

6.6. Examples of application

Question 1.

1. Why is it more difficult to develop antiviral drugs than antibiotics?
2. Does the binding of the antiviral to the capsid proteins allow indirect inhibition of uncoating?

Antimicrobial agents constitute a diverse set of substances and processes essential for the prevention and control of microbial infections. They can be of chemical origin, such as antibiotics, antiseptics, and disinfectants, or of physical origin, such as heat, cold, radiation, and filtration. Each type of agent has specific characteristics that determine its spectrum of activity, mechanism of action, and appropriate use.

Understanding fundamental concepts such as decimal reduction time (D), MIC (Minimum Inhibitory Concentration), and MBC (Minimum Bactericidal Concentration) allows for quantifying microbial resistance and adapting elimination or inhibition protocols. These parameters are particularly important for physical processes, where effectiveness depends more on exposure time and treatment intensity than on simple concentration.

The classification of antimicrobial agents according to their chemical relatedness, mechanism of action, and usage facilitates rational selection. For example, grouping antibiotics by families allows predicting their spectrum of activity and understanding potential resistance mechanisms. Similarly, distinguishing antiseptics from disinfectants and detergents helps select the appropriate agent based on the type of surface or tissue being treated.

Finally, mastering antimicrobial agents is crucial for public health safety, the prevention of nosocomial infections, and the protection of food and the environment. Judicious use, combined with knowledge of the properties of these agents, helps limit the development of microbial resistance, improve treatment effectiveness, and ensure public health.

Thus, this course material aims not only to provide fundamental knowledge on antimicrobial agents, but also to develop students' critical thinking skills regarding their appropriate use and the challenges of microbial resistance. In a context of increasing emergence of resistant microorganisms, understanding antimicrobial mechanisms and their applications represents a major challenge for public health protection.

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