Microbiology

Unit 5 · Control of Microorganisms

Every autoclave run, every bottle of hand sanitiser, every antibiotic prescription and every packet of pickle is a way of controlling microbes. After this unit you can choose a sterilization or disinfection method and justify it, calculate a heat process from D- and z-values, explain where each antibiotic class acts and how bacteria resist it, and say why a food spoils and which hurdles keep it safe.

Taught at 8 of 10 | Usually Sem 3 | 5 chapters

About this unit

This unit is about stopping microbes: killing them, removing them or keeping them from growing. It starts outside the body with physical methods such as heat, filters and radiation (Chapter 5.1) and chemical methods such as disinfectants and antiseptics (5.2). It then moves inside the body with antibiotics and the targets they hit (5.3), and with the way bacteria fight back through resistance (5.4). The last chapter (5.5) applies all of it to food: why food spoils and how preservation keeps it safe. The kill-rate ideas of Chapter 5.1 (D-value, z-value) come back in canning in 5.5, and the drug targets of 5.3 are the starting point for every resistance mechanism in 5.4.

Chapters

1. Physical control: heat, filtration and radiation
2. Chemical control: disinfectants and antiseptics
3. Antibiotics and how they act
4. Antimicrobial resistance
5. Microbial spoilage of food and its control

How to use this book

- Read the lead paragraph and the figure of each chapter first; they carry the one idea.
- Cover the solution of the worked example and do it yourself with a pencil, then compare step by step.
- Answer each "Check yourself" question in your head or on paper before you open it.

5.1 Physical control: heat, filtration and radiation

Physical control means killing or removing microbes with energy or a barrier instead of a chemical: steam in an autoclave, a hot air oven, a membrane filter, a UV lamp or a gamma source. Every microbiology lab, hospital and food factory depends on it, because no culture, injection or canned food is safe unless the unwanted microbes are gone first. The skill this chapter builds is choosing the right method for the material and knowing how long it must run.

You need: the bacterial endospore and cell wall (Unit 2, Bacterial morphology and cell structure) and culture media (Unit 4, Nutritional requirements and culture media).

Words first: how much is "clean"?

Sterilization destroys or removes all living forms, including bacterial endospores. It is absolute: an object is sterile or it is not. **Disinfection** kills most disease-causing microbes on an object or surface, but not all spores. **Antisepsis** is disinfection of living tissue such as skin. **Sanitization** lowers microbial numbers to a level public-health rules accept, as in a restaurant dishwasher.

A suffix tells you what an agent does. **-cidal** agents kill (bactericidal, sporicidal, virucidal). **-static** agents only stop growth (bacteriostatic); remove the agent and growth starts again. Refrigeration is bacteriostatic; autoclaving is cidal.

How microbes die: D-value and z-value

When a population is heated at a constant temperature, it does not die all at once. In each minute, the same fraction of the survivors dies. So the number of survivors falls by a constant factor per unit time, and a plot of $\log_{10}$(survivors) against time is a straight line. This is **first-order** (logarithmic) death:

$$N = N_0 \times 10^{(-t / D)} \quad \text{or} \quad \log_{10} N = \log_{10} N_0 - t / D$$

Here N_0 is the starting number of cells or spores, N is the number alive after time t, and **D** (decimal reduction time) is the time at a stated temperature that kills 90% of the population, that is, one log cycle. D is written with its temperature, for example $D_{121} = 1.5$ min.

The **z-value** is the rise in temperature that makes D ten times smaller. For many bacterial spores z is close to 10 °C, so the D at a lower temperature T is $D_T = D_{ref} \times 10^{((T_{ref} - T)/z)}$. The **F-value** is the total lethality of a process, expressed as minutes at a reference temperature; F_0

means minutes at 121.1 °C with z = 10 °C, and Chapter 5.5 uses it for canning. Older books use **thermal death point** and **thermal death time**; both depend on the starting number of cells, which is why D replaced them.

Killing never reaches exactly zero, so a process aims at a **sterility assurance level** (SAL); medical devices are usually sterilized to 10^{-6}, a one-in-a-million chance that an item carries a survivor. A dirtier load (bigger N_o) needs a longer run.

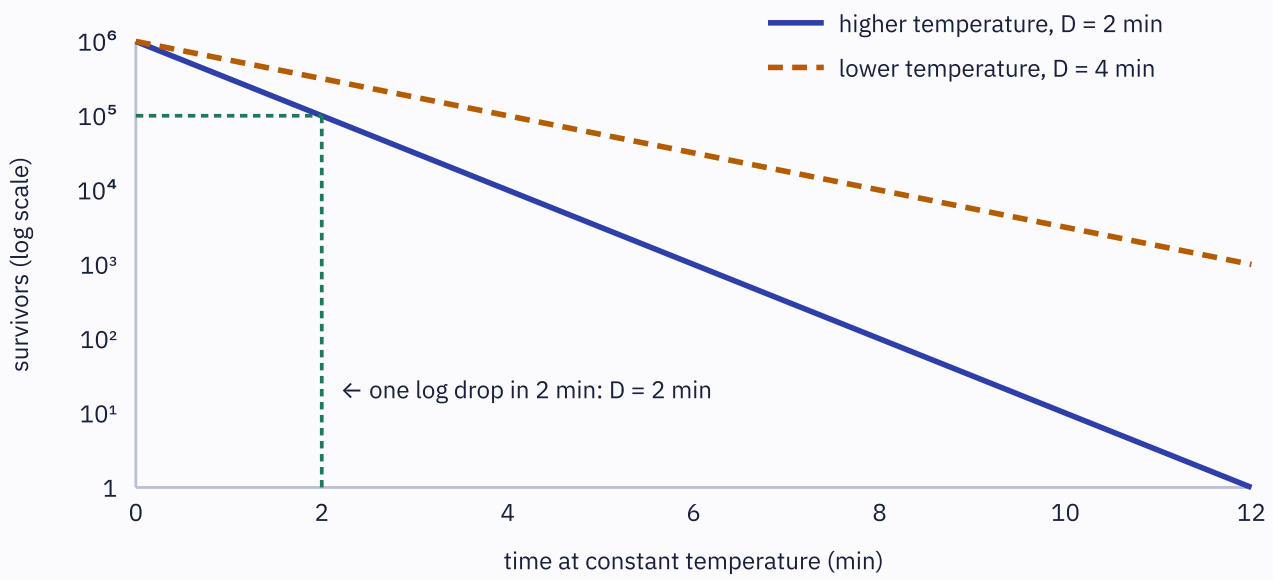


Figure 5.1.1 Survivor curves for 10^6 spores. On a log scale the deaths fall on straight lines; the time for one log drop is the D-value. Notice that the cooler process (dashed) needs twice as long for each log, and neither line ever touches zero.

Heat: moist and dry

Moist heat kills by denaturing and coagulating proteins, and water makes proteins unfold at lower temperatures. Steam also gives up its latent heat when it condenses on a cold object, so it heats the load fast. That is why steam at 121 °C works in minutes while dry air at the same temperature takes hours.

- **Autoclave**: saturated steam at about 15 psi (about 103 kPa) above atmospheric pressure, which raises the boiling point to 121 °C. Textbooks quote 15 minutes for small unwrapped loads; the US CDC guideline gives 30 minutes at 121 °C for wrapped instruments in a gravity-displacement autoclave, and 4 minutes at 132 °C in a prevacuum autoclave. Air must be driven out first, because an air pocket stays cooler than steam. Used for media, instruments and discarded cultures.
- **Boiling** at 100 °C for 10 to 30 minutes kills vegetative cells and most viruses, but not all endospores. It disinfects; it does not sterilize.

- **Tyndallization** (intermittent sterilization): steaming at 100 °C for about 30 minutes on three successive days. Spores that survive day one germinate overnight and the young vegetative cells die the next day. Used for media that 121 °C would spoil.
- **Pasteurization**: mild heat that kills pathogens and lowers spoilage organisms in milk and juice without cooking them. The batch (LTLT) method is 63 °C for 30 minutes; the flash (HTST) method is 72 °C for 15 seconds.

Dry heat kills mainly by oxidation and needs higher temperatures for longer. The **hot air oven** runs at 160 °C for 2 hours or 170 °C for 1 hour, for glassware, metal instruments, oils and powders that steam cannot reach or would damage. **Incineration** burns waste to ash, and **flaming** an inoculation loop red-hot sterilizes it in seconds.

A cycle is checked with indicators. Chemical indicator tape changes colour when heated, which proves the temperature was reached but not that spores died. A **biological indicator** is a strip of resistant spores, *Geobacillus stearothermophilus* for steam, incubated after the run; no growth means the cycle worked.

Filtration and radiation

Filtration removes microbes instead of killing them, so it suits heat-sensitive liquids such as antibiotic solutions, vitamins, serum and enzyme solutions. A **membrane filter** (cellulose ester or polycarbonate) with 0.22 µm pores holds back bacteria; 0.45 µm membranes are used to collect bacteria from water for counting. Viruses and some mycoplasmas pass through 0.22 µm pores, so a filtered liquid is not strictly sterile for them. Air is filtered by **HEPA** filters, which trap at least 99.97% of 0.3 µm particles; they supply laminar airflow hoods, biosafety cabinets and operating theatres.

Ionizing radiation (gamma rays from cobalt-60, electron beams, X-rays) knocks electrons off water molecules and makes hydroxyl radicals that break DNA. It penetrates packaging, so plastic syringes, catheters, gloves, sutures and Petri plates are sterilized after sealing, without heat; this is called cold sterilization. **Ultraviolet** light near 260 nm, where DNA absorbs most strongly, joins neighbouring pyrimidines into dimers (mostly thymine dimers) that block replication; germicidal lamps emit at 254 nm. UV does not penetrate glass, paper or thick liquid, so it only treats air, surfaces and thin films of water.

Cold, drying and high salt or sugar mostly stop growth rather than kill; they return as food preservation in Chapter 5.5.

WORKED EXAMPLE

Question (8 marks). One litre of medium carries 10^5 spores per mL. For these spores D_{121} = 1.5 min and z = 10 °C. (a) How long at 121 °C reduces the load to one surviving spore? (b) How long gives a sterility assurance level of 10^{-6}? (c) If the autoclave only reaches 115 °C, how long is needed for (b)?

1. Starting load: $N_0 = 10^5$ spores/mL × 1000 mL = 10^8 spores, so $\log_{10}N_0 = 8$.
2. (a) Reaching N = 1 (log 0) needs 8 log reductions. Time = 8 × D = 8 × 1.5 = **12 min**.
3. (b) SAL 10^{-6} means $\log_{10}N = -6$, so 8 − (−6) = 14 log reductions. Time = 14 × 1.5 = **21 min**.
4. (c) D at 115 °C: D_{115} = 1.5 × 10^((121 − 115)/10) = 1.5 × 10^0.6 = 1.5 × 3.98 = 5.97 min.
5. Time = 14 × 5.97 = **83.6 min**, about four times longer for a 6 °C drop.

These times start only when the centre of the flask reaches the set temperature, so large loads need longer cycles.

EXAM TRAPS

- Pressure does not kill; it only raises the boiling point so steam can reach 121 °C. An autoclave full of trapped air at 15 psi is cooler and fails.
- Boiling and pasteurization are not sterilization. Spores survive both.
- D is the time for a 90% kill, not 100%. A 99.9% kill is 3D.
- Filtration removes, it does not kill; and 0.22 µm filters let viruses through.
- UV has poor penetration; gamma rays penetrate. Do not swap them in "sterilization of disposable syringes" answers.

CHECK YOURSELF

– REMEMBER **State the standard conditions for a hot air oven and for an autoclave.** 2 marks

Hot air oven: 160 °C for 2 h (or 170 °C for 1 h). Autoclave: saturated steam at 121 °C, about 15 psi above atmospheric, 15 min for small loads (longer for wrapped or large loads).

– REMEMBER **Which organism's spores are used as the biological indicator for steam sterilization?** 1 mark

Geobacillus stearothermophilus.

– UNDERSTAND **Why does moist heat sterilize faster than dry heat at the same temperature?** 3 marks

Water helps proteins unfold and coagulate at lower temperatures, and condensing steam releases latent heat that warms the load quickly. Dry heat kills by slow oxidation and air transfers heat poorly.

– UNDERSTAND **Explain the principle of tyndallization.** 3 marks

Steaming at 100 °C kills vegetative cells but not spores. During overnight incubation surviving spores germinate into heat-sensitive vegetative cells, which the next day's steaming kills. Three cycles leave the medium sterile without exposing it to 121 °C.

– APPLY **A culture has 10^7 cells; D_{72} = 0.5 s. How many survive 15 s at 72 °C?** 3 marks

Log reductions = 15/0.5 = 30. $\log_{10}N = 7 - 30 = -23$, so $N = 10^{-23}$: effectively none survive.

– APPLY **Choose a method to sterilize (i) a vitamin solution, (ii) plastic Petri plates in sealed packs, (iii) glass pipettes.** 3 marks

(i) Membrane filtration through 0.22 µm. (ii) Gamma or electron-beam irradiation. (iii) Hot air oven, 160 °C for 2 h.

– ANALYZE **An autoclave run's indicator tape changed colour but the spore strip grew. Give two likely causes.** 4 marks

The tape only shows the surface got hot. Likely causes: air was not removed, so the load centre stayed below 121 °C; the cycle was too short for a large or wrapped load; or the load was packed too tightly for steam to reach it.

BEYOND THE SYLLABUS

Industry validates sterilization against international standards: ISO 17665 for moist heat and ISO 11137 for radiation sterilization of health care products, both built on the D-value and SAL ideas above. Heat-sensitive devices are often treated with hydrogen peroxide gas plasma, and UV-C LEDs are replacing mercury lamps for water disinfection. Search "sterility assurance level", "overkill method" and "bioburden method" to see how a cycle is designed. This is not for the exam.

5.2 Chemical control: disinfectants and antiseptics

Chemical control uses substances that kill or inhibit microbes on surfaces, instruments, water and skin, where heat cannot be used. The Dettol in a hospital mop bucket, bleaching powder in a village well, 70% alcohol on a vaccination swab and glutaraldehyde for an endoscope are all chemical agents chosen for one job. Each class attacks a different part of the cell, and each fails in a predictable way, which is what examiners ask about.

You need: kill kinetics and the D-value from Chapter 5.1; cell membranes and walls (Unit 2, Bacterial cell wall: Gram-positive and Gram-negative).

Terms and how to choose an agent

A **disinfectant** is used on non-living objects; it may be too harsh for tissue. An **antiseptic** is mild enough for skin and mucous membranes. A **sanitizer** brings counts down to a safe public-health level, as on food-contact surfaces. A **sterilant** (chemosterilant) kills spores too, given enough time. The general word **germicide** covers all of them.

Hospitals pick the level of treatment from what the item touches (the **Spaulding classification**). Critical items enter sterile tissue or blood, such as surgical instruments and needles, and must be sterile. Semicritical items touch mucous membranes, such as endoscopes, and need high-level disinfection. Noncritical items touch only intact skin, such as BP cuffs and bed rails, and need low-level disinfection.

Microbes are not equally easy to kill. Figure 5.2.1 ranks them. An agent that kills a group also kills every group below it, so an alcohol that kills mycobacteria also kills vegetative bacteria and enveloped viruses.

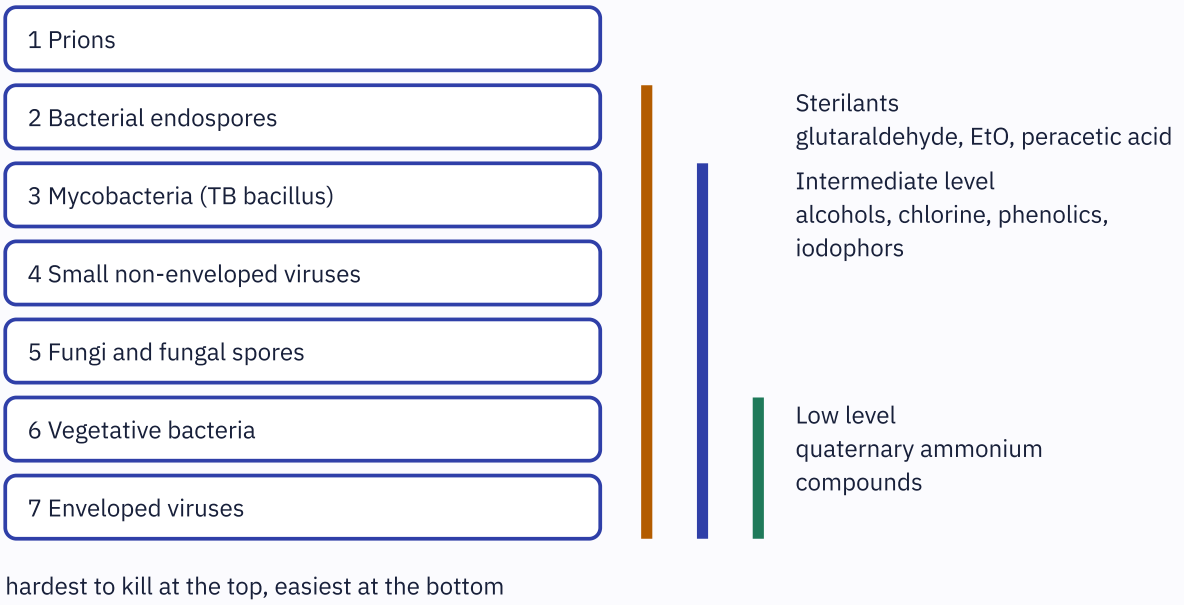


Figure 5.2.1 Resistance ladder for chemical agents. Each bar starts at the hardest group that class of agent can kill and covers everything below. Notice that only sterilants reach spores, and prions sit above every routine agent.

What affects a disinfectant

- **Concentration and contact time.** Killing follows the same first-order pattern as heat (Chick's law). Watson added the effect of concentration: $C^n \times t = K$, where C is concentration, t the time for a given kill, K a constant and n the **concentration exponent**. A large n means dilution weakens the agent sharply; for phenol n is about 6.
- **Organic matter.** Blood, pus, faeces and food residues react with chlorine, iodine and quaternary ammonium compounds and use them up. Clean first, then disinfect.
- **Temperature and pH.** Warmer usually works faster. Chlorine is far stronger at slightly acidic pH, because the killing form, hypochlorous acid (HOCl), turns into weaker hypochlorite ion (OCl^-) as pH rises.
- **Number and state of microbes.** More cells take longer; biofilms (Unit 6, Biofilms) and dried films are protected.

The main classes

CLASS	EXAMPLES	HOW IT ACTS	TYPICAL USE AND LIMITS
Phenolics	phenol, cresols, chloroxylenol (Dettol)	damage membranes, denature proteins	floors, discarded cultures; work in organic matter; irritate skin. Lister used phenol (carbolic acid) in surgery in the 1860s.

CLASS	EXAMPLES	HOW IT ACTS	TYPICAL USE AND LIMITS
Alcohols	ethanol, isopropanol, 60 to 90% in water	denature proteins, dissolve membrane lipids	skin before injection, hand rubs, thermometers; need water to work, evaporate fast, do not kill spores.
Halogens	sodium hypochlorite (bleach), bleaching powder, chloramines; tincture of iodine, povidone-iodine	oxidize proteins and other cell parts	drinking water, blood spills, surgical skin preparation; chlorine is used up by organic matter.
Biguanides	chlorhexidine	disrupt membranes	surgical hand scrub, mouthwash; Savlon combines it with cetrimide.
Quaternary ammonium compounds	benzalkonium chloride, cetrimide	cationic detergents that disrupt membranes	floors, food-contact surfaces; stronger against Gram-positives; inactivated by soap and organic matter; some *Pseudomonas* grow in weak solutions.
Heavy metals	silver nitrate, silver sulfadiazine, copper sulfate	bind thiol (–SH) groups of proteins	burn dressings, algicide in tanks; tiny amounts act (oligodynamic action).
Aldehydes	2% glutaraldehyde, formaldehyde	alkylate amino and other groups of proteins and nucleic acids	endoscopes, fumigation of rooms; slow, toxic, irritant.
Gas	ethylene oxide (EtO)	alkylates proteins and DNA	sterilizes heat-sensitive plastics and devices; penetrates packs; toxic, explosive, needs aeration.
Peroxygens	hydrogen peroxide, peracetic acid, ozone	oxidize via reactive oxygen	wounds (3% H_2O_2), sterilant for devices, water; catalase-producing bacteria resist weak H_2O_2.

Soap is not in the table because it mainly removes microbes: it lifts oil and dirt so that rinsing carries cells away, which is called degerming.

Testing how good a disinfectant is

The **phenol coefficient** compares an agent with phenol against a standard organism. In the **Rideal–Walker test**, the test organism is *Salmonella* Typhi; dilutions of the agent and of phenol are inoculated, sampled at fixed minutes and cultured. The phenol coefficient is the highest dilution of the agent that kills in a stated time divided by the same dilution for phenol. The

Chick–Martin test adds organic matter (yeast) to mimic real use. Because a single number hides the effect of dirt and surfaces, modern labs prefer the **use-dilution test**, where bacteria dried on steel carriers are dipped in the recommended dilution, and **in-use tests**, where samples from disinfectant buckets in the ward are cultured to see if anything survives.

WORKED EXAMPLE

Question (6 marks). In a phenol coefficient test against *S.* Typhi at 20 °C, phenol at 1:90 killed the organism in 10 minutes but not in 5 minutes. A new disinfectant X did the same at 1:400. (a) Find the phenol coefficient of X. (b) For phenol, n = 6. If a 1% phenol solution kills in 10 minutes, how long will a 0.5% solution take?

1. (a) Phenol coefficient = dilution of X ÷ dilution of phenol for the same result = 400 ÷ 90 = **4.4**. X is about four times as effective as phenol in this test.
2. (b) Watson: $C_1^n\, t_1 = C_2^n\, t_2$, so $t_2 = t_1 \times (C_1/C_2)^n$.
3. $C_1/C_2 = 1/0.5 = 2$, and $2^6 = 64$.
4. $t_2 = 10$ min × 64 = **640 min**, more than ten hours.

Halving phenol's strength makes it 64 times slower. This is why a ward that "saves" disinfectant by over-diluting it ends up with buckets in which bacteria survive.

EXAM TRAPS

- Disinfectant is for objects, antiseptic is for living tissue. The same chemical can be both at different strengths.
- Absolute (100%) alcohol is weaker than 70%; water is needed to denature proteins.
- A phenol coefficient above 1 means stronger than phenol, but it says nothing about performance in blood or on a dirty floor.
- Glutaraldehyde and ethylene oxide are sterilants; alcohol and quaternary ammonium compounds are not, because they do not kill spores.
- Do not write "chlorine is better at high pH". HOCl, the active form, dominates at slightly acidic pH.

CHECK YOURSELF

– REMEMBER **Name one disinfectant from each of: halogens, aldehydes, quaternary ammonium compounds.** 3 marks

Halogens: sodium hypochlorite or povidone-iodine. Aldehydes: glutaraldehyde or formaldehyde. Quaternary ammonium: benzalkonium chloride or cetrimide.

– REMEMBER **Which test organism is used in the Rideal–Walker test?** 1 mark

Salmonella Typhi.

– UNDERSTAND **Why must a blood spill be cleaned before it is disinfected with bleach?** 2 marks

Organic matter reacts with chlorine and uses it up, so little active HOCl is left to kill microbes; the protein also shields cells. Removing it first lets the bleach act.

– UNDERSTAND **Explain the Spaulding classification with one example of each class.** 4 marks

Critical items enter sterile tissue (scalpel): sterilize. Semicritical items touch mucous membranes (endoscope): high-level disinfection. Noncritical items touch intact skin (BP cuff): low-level disinfection.

– APPLY **An agent has n = 1. A 2% solution kills in 20 min. How long will 1% take?** 2 marks

$t_2 = 20 \times (2/1)^1 = 40$ min. With n = 1, halving strength only doubles the time.

– APPLY **Choose an agent for: (i) a heat-sensitive plastic catheter in its pack, (ii) skin before a blood draw, (iii) a village well.** 3 marks

(i) Ethylene oxide gas (or gamma radiation). (ii) 70% alcohol. (iii) Bleaching powder (chlorination).

– ANALYZE **A hospital finds *Pseudomonas* growing in its benzalkonium chloride bottles. Explain how and suggest a fix.** 4 marks

Quaternary ammonium compounds are weaker against Gram-negatives, whose outer membrane slows entry; over-dilution, topping up old bottles and organic contamination let resistant *Pseudomonas* survive and grow. Fix: fresh solutions at the right strength, no topping up, clean containers, and in-use testing.

BEYOND THE SYLLABUS

Regulators now test disinfectants with European EN standards and US EPA methods instead of the phenol coefficient, and they demand claims per organism and contact time. Triclosan was withdrawn from many consumer soaps after concerns about safety and resistance, and quaternary ammonium compounds are watched for the same reason. Hydrogen peroxide vapour is used to decontaminate whole hospital rooms and pharmaceutical cleanrooms. Search "EN 1276 suspension test" and "hydrogen peroxide vapour decontamination" to read further. This is not for the exam.

5.3 Antibiotics and how they act

An antibiotic is a drug that stops bacteria inside the body without harming the patient much. It can do this only by hitting something bacteria have and human cells lack, or have in a different form: the peptidoglycan wall, the 70S ribosome, bacterial DNA gyrase, or the enzymes that make folic acid. Knowing the five target sites lets you predict which drug works on which organism, why some drugs are toxic, and, in Chapter 5.4, how bacteria escape them.

You need: peptidoglycan and the two kinds of cell wall (Unit 2, Bacterial cell wall: Gram-positive and Gram-negative); disinfectants for comparison (Chapter 5.2).

Key terms and a short history

Strictly, an **antibiotic** is a substance made by a microbe that, in small amounts, inhibits or kills other microbes; Selman Waksman popularised the word. Many drugs today are **semi-synthetic** (a natural core modified in the lab, like ampicillin) or fully synthetic (sulfonamides, fluoroquinolones). **Antimicrobial agent** or **chemotherapeutic agent** covers all of them, including antifungals and antivirals.

Selective toxicity, the idea that a drug can harm the microbe and spare the host, came from Paul Ehrlich's search for a "magic bullet", which gave Salvarsan against syphilis. Alexander Fleming saw a *Penicillium* mould kill *Staphylococcus* colonies in 1928; Howard Florey and Ernst Chain made penicillin a usable drug during the Second World War. Gerhard Domagk showed the sulfonamide dye Prontosil worked in infections in the 1930s, and Waksman's group found streptomycin, the first drug against tuberculosis, in soil *Streptomyces*. *Streptomyces* species remain the largest source of natural antibiotics (tetracyclines, chloramphenicol, streptomycin).

A drug's **spectrum** is the range of bacteria it acts on: **narrow** (penicillin G mainly against Gram-positives) or **broad** (tetracyclines against Gram-positives, Gram-negatives, rickettsiae and chlamydiae). A **bactericidal** drug kills; a **bacteriostatic** drug stops growth and relies on the immune system to clear the infection.

The five target sites

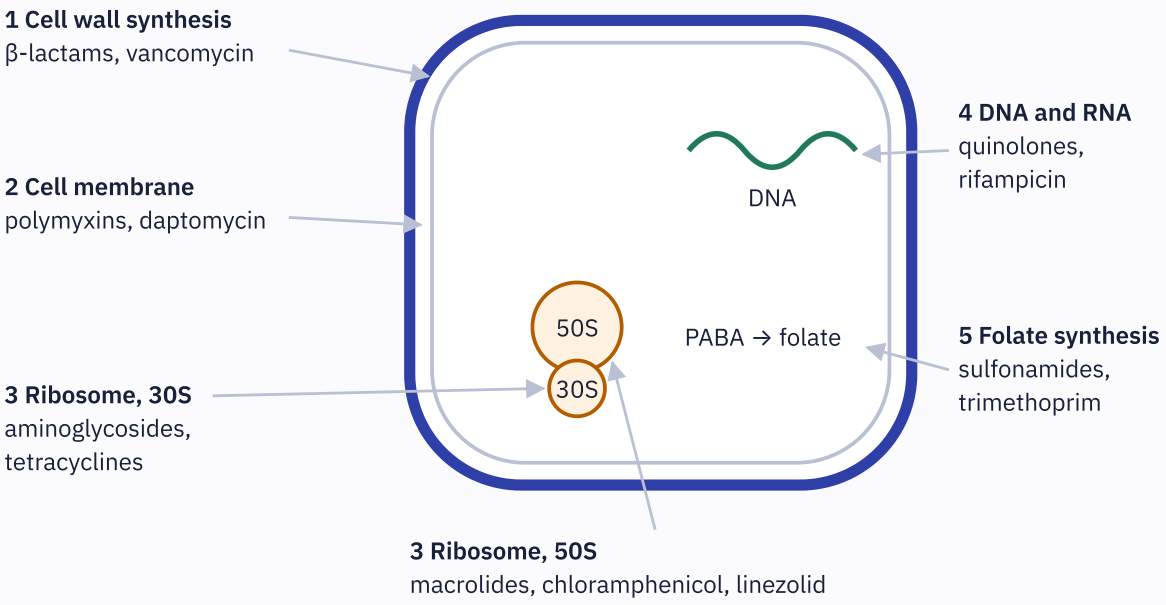


Figure 5.3.1 Where the main antibiotic classes act on a bacterial cell. Each target is either absent from human cells (peptidoglycan, the folate-making enzymes) or built differently there (80S ribosomes, human topoisomerases). Notice that one ribosome holds two targets: the 30S and the 50S subunits.

1. **Cell wall synthesis.** β-lactams (penicillins, cephalosporins, carbapenems, monobactams) contain a four-membered β-lactam ring. They bind the **penicillin-binding proteins** (PBPs), the transpeptidases that cross-link peptidoglycan strands. The weakened wall bursts under osmotic pressure as the cell grows, so these drugs are bactericidal and act best on dividing cells. **Vancomycin** (a glycopeptide) binds the D-alanyl-D-alanine end of the wall precursor and blocks its assembly; it is too large to cross the Gram-negative outer membrane, so it is used against Gram-positives such as MRSA. Isoniazid blocks mycolic acid synthesis in the TB bacillus.
2. **Cell membrane.** Polymyxin B and colistin bind lipopolysaccharide and disrupt the membranes of Gram-negatives; daptomycin acts on Gram-positive membranes. Membranes are similar in all cells, so these drugs are more toxic, especially to the kidney. Antifungals work here too: amphotericin B binds ergosterol and azoles block its synthesis (fungal diseases are in Unit 2).

3. **Protein synthesis.** Bacteria have 70S ribosomes (30S + 50S subunits); human cytoplasm has 80S. On the 30S subunit, **aminoglycosides** (streptomycin, gentamicin) cause misreading of mRNA and are bactericidal, while **tetracyclines** block aminoacyl-tRNA from binding the A site and are bacteriostatic. On the 50S subunit, **macrolides** (erythromycin, azithromycin) block the exit tunnel for the growing peptide, **chloramphenicol** inhibits peptidyl transferase, and **linezolid** prevents the initiation complex from forming. Human mitochondria have bacteria-like ribosomes, which explains some side effects.
4. **Nucleic acid synthesis. Fluoroquinolones** (ciprofloxacin, levofloxacin) inhibit DNA gyrase and topoisomerase IV, the enzymes that relieve supercoiling during replication. **Rifampicin** binds the β subunit of bacterial RNA polymerase and blocks transcription; it is a first-line TB drug. **Metronidazole** is activated only inside anaerobes and damages their DNA.
5. **Folate synthesis (antimetabolites).** Bacteria must make folic acid from para-aminobenzoic acid (PABA); humans take folate from food. **Sulfonamides** look like PABA and inhibit dihydropteroate synthase. **Trimethoprim** inhibits dihydrofolate reductase one step later. Together, as **co-trimoxazole**, they block two steps of one pathway and act more strongly than either alone (synergy).

Measuring susceptibility: MIC, MBC and the disk test

The **minimum inhibitory concentration** (MIC) is the lowest drug concentration that prevents visible growth after overnight incubation. It is found by **broth dilution**: a row of tubes or microplate wells with twofold dilutions of the drug, each inoculated with the same number of bacteria. The **minimum bactericidal concentration** (MBC) is the lowest concentration that kills 99.9% of the inoculum, found by plating the clear tubes on drug-free agar. When MBC ÷ MIC is 4 or less, the drug is usually called bactericidal for that strain.

In the **Kirby–Bauer disk diffusion** test, a lawn of bacteria is spread on Mueller–Hinton agar and paper disks holding a fixed amount of drug are placed on it. The drug diffuses out, and a clear **zone of inhibition** forms. The zone diameter is compared with tables of breakpoints (from CLSI or EUCAST) to report the strain as susceptible, intermediate or resistant. A zone diameter cannot be compared across drugs, because each drug diffuses differently. The **E-test** strip carries a gradient of drug and gives the MIC directly where the ellipse of inhibition meets the strip.

WORKED EXAMPLE

Question (6 marks). A clinical *E. coli* was tested by broth dilution with an antibiotic. After 18 h at 37 °C the tubes looked like this. Each clear tube was then plated on drug-free agar. Find the MIC and MBC and say whether the drug is bactericidal for this strain.

DRUG (MG/ML)	64	32	16	8	4	2	1	0 (CONTROL)
Broth after 18 h	clear	clear	clear	clear	clear	turbid	turbid	turbid
Colonies on subculture	none	none	none	many	many	—	—	—

1. The control tube is turbid, so the organism grew and the test is valid.
2. MIC = lowest concentration with no visible growth = **4 µg/mL**.
3. MBC = lowest concentration with no survivors on subculture = **16 µg/mL** (the 8 and 4 µg/mL tubes were inhibited but still held live cells).
4. MBC ÷ MIC = 16 ÷ 4 = 4, so by the usual rule the drug is **bactericidal** for this strain, just at the limit.
5. Whether 4 µg/mL counts as "susceptible" depends on the breakpoint for that drug and organism in CLSI or EUCAST tables, not on the number alone.

EXAM TRAPS

- Penicillin does not dissolve existing walls; it stops new cross-links, so it needs growing cells.
- Aminoglycosides and tetracyclines both bind 30S, but aminoglycosides kill and tetracyclines only inhibit.
- Sulfonamides inhibit dihydropteroate synthase; trimethoprim inhibits dihydrofolate reductase. Students often swap them.
- A bigger zone for drug A than drug B does not mean A is better; compare each zone only with its own breakpoint.
- Antibiotics do not act on viruses; they have no wall, ribosome or folate pathway of their own.

CHECK YOURSELF

– REMEMBER **Define MIC and MBC.** 2 marks

MIC: lowest concentration that prevents visible growth. MBC: lowest concentration that kills 99.9% of the inoculum.

– REMEMBER **Name the target of rifampicin and of ciprofloxacin.** 2 marks

Rifampicin: β subunit of bacterial RNA polymerase. Ciprofloxacin: DNA gyrase and topoisomerase IV.

– UNDERSTAND **Explain selective toxicity with two examples.** 4 marks

A drug harms the microbe more than the host because it hits a target the host lacks or has in a different form. Penicillin attacks peptidoglycan, absent in human cells; sulfonamides block folate synthesis, which humans do not perform because they take folate from food.

– UNDERSTAND **Why does vancomycin not work on most Gram-negative bacteria?** 2 marks

It is a large glycopeptide that cannot cross the Gram-negative outer membrane to reach the peptidoglycan precursors.

– APPLY **Twofold dilutions from 32 to 0.5 µg/mL: growth at 1 and 0.5 only; subculture shows no colonies from 32, 16 and 8, colonies from 4 and 2. Find MIC and MBC.** 3 marks

MIC = 2 µg/mL; MBC = 8 µg/mL; ratio 4, so bactericidal by the usual rule.

– ANALYZE **Why are sulfonamide and trimethoprim given together?** 4 marks

They block two steps of the same folate pathway (dihydropteroate synthase and dihydrofolate reductase). Sequential blockade gives synergy: lower doses work, the combination can be bactericidal, and a mutant must resist both to survive.

BEYOND THE SYLLABUS

Few new antibiotic classes have reached patients in recent decades, so research looks at uncultured soil bacteria, genome mining of *Streptomyces* gene clusters, and machine-learning screens of chemical libraries. Combination drugs that pair a β-lactam with a β-lactamase inhibitor extend old drugs, and bacteriophage therapy is tried for infections that no drug can treat. Automated systems such as broth microdilution panels now report MICs in clinical labs within a day. Search "biosynthetic gene cluster mining" and "antibiotic discovery pipeline" to go further. This is not for the exam.

5.4 Antimicrobial resistance

Antimicrobial resistance (AMR) is the ability of a microbe to survive a drug that used to kill or stop it. It is evolution you can watch in a hospital: every dose kills the sensitive cells and leaves the resistant ones to multiply, and resistance genes jump between species on plasmids. A large global study published in *The Lancet* in 2022 estimated that in 2019 about 1.27 million deaths were directly caused by resistant bacterial infections, and about 4.95 million deaths were associated with them. This chapter explains how bacteria resist, how resistance spreads, and what slows it down.

You need: the drug targets of Chapter 5.3; plasmids and gene transfer (Unit 4, Bacterial recombination: transformation, transduction, conjugation).

Kinds of resistance

Intrinsic resistance is natural to a species because it lacks the target or the drug cannot get in. *Mycoplasma* has no cell wall, so β-lactams cannot act on it (Unit 2, Mycoplasma and actinomycetes). The outer membrane of Gram-negatives keeps out vancomycin. Strict anaerobes resist aminoglycosides, whose uptake needs oxygen-dependent transport.

Acquired resistance appears in a species that was once susceptible, by **mutation** in the cell's own genes or by **gaining a resistance gene** from another cell. A strain is called **multidrug-resistant** (MDR) when it resists several classes of drug.

Do not confuse resistance with **tolerance**. **Persister** cells are a small dormant fraction of a population that survives a drug because it is not growing, and cells inside a **biofilm** (Unit 6,

Biofilms) are shielded by their matrix. Their offspring are still susceptible, because nothing in the genome changed.

Four ways bacteria resist

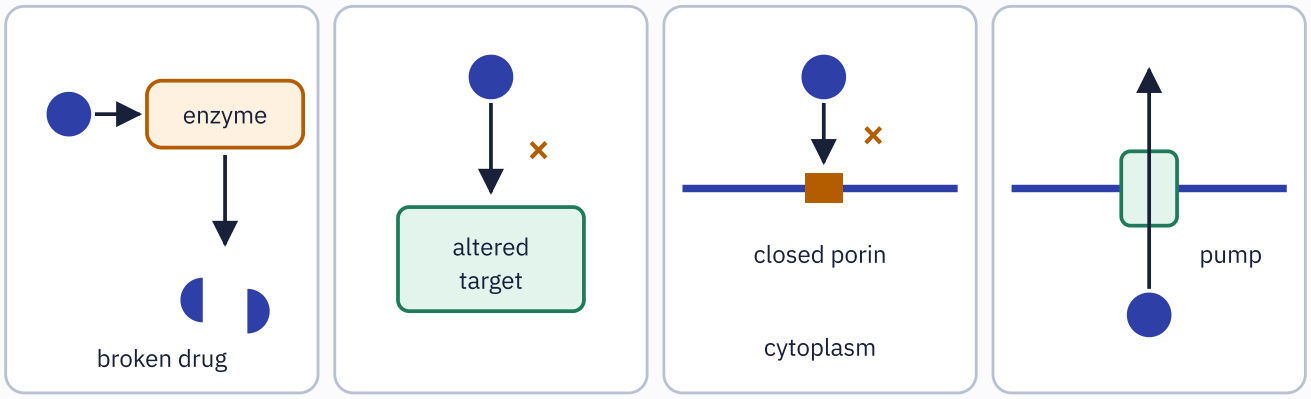


Figure 5.4.1 The four main resistance mechanisms; the blue circle is the drug. Panels 3 and 4 show the cell envelope as a blue line with the outside above it. Notice that only mechanism 1 destroys the drug; the other three keep it from reaching or binding its target.

1. **Enzymatic inactivation. β-lactamases** cut the β-lactam ring and destroy penicillins and their relatives. **Extended-spectrum β-lactamases** (ESBLs) also destroy newer cephalosporins, and **carbapenemases** destroy carbapenems, the drugs kept for the worst infections. NDM-1 (New Delhi metallo-β-lactamase), first described in 2009, is a carbapenemase spread on plasmids among *Klebsiella*, *E. coli* and other Gram-negatives. Other enzymes add chemical groups to aminoglycosides (acetyl, phosphate or adenylyl) or acetylate chloramphenicol, so the drug no longer binds.
2. **Target modification.** In **MRSA** (methicillin-resistant *Staphylococcus aureus*), the *mecA* gene makes PBP2a, a penicillin-binding protein that β-lactams bind poorly, so cross-linking continues. In **VRE** (vancomycin-resistant enterococci), *van* genes make wall precursors end in D-Ala-D-lactate instead of D-Ala-D-Ala, which vancomycin binds far less tightly. Methylation of 23S rRNA by *erm* genes blocks macrolides. Point mutations in DNA gyrase cause fluoroquinolone resistance, and mutations in the *rpoB* gene for RNA polymerase cause rifampicin resistance in *Mycobacterium tuberculosis*.
3. **Reduced entry.** Gram-negatives take in many drugs through porin channels in the outer membrane. Losing or narrowing a porin, such as OprD in *Pseudomonas aeruginosa*, lowers entry of carbapenems like imipenem.

4. **Efflux.** Membrane pumps push the drug out faster than it comes in. Tet pumps export tetracyclines; multidrug pumps such as AcrAB-TolC in *E. coli* export several classes at once, giving MDR in one step.

A cell can also make a **bypass**: a second, drug-insensitive enzyme. Plasmid *sul* and *dfr* genes encode resistant dihydropteroate synthase and dihydrofolate reductase, so the folate pathway keeps running under sulfonamide and trimethoprim.

How resistance spreads

Vertical spread: a rare mutant survives the drug and its offspring take over. The drug does not cause the mutation; it selects a mutant that was already there. **Horizontal spread**: genes move between cells, even between species. **R plasmids** (resistance plasmids) carry several resistance genes and move by **conjugation**; **transposons** jump genes between plasmid and chromosome; **integrons** collect resistance gene cassettes behind one promoter. Transformation and transduction add more routes. Horizontal transfer explains why one plasmid can make a strain resistant to five drug classes overnight.

The pressure comes from use. Antibiotics taken for viral fevers, sold without prescription, given in wrong doses, or used to make poultry and fish grow faster all select resistant bacteria. Hospitals concentrate sick patients, devices and heavy antibiotic use, so resistant strains spread there first; poor sanitation and untreated effluent carry them into rivers and soil.

The most feared hospital pathogens are grouped as **ESKAPE**: *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* species.

Slowing it down

- **Stewardship**: right drug, right dose, right duration, chosen after a culture and susceptibility test (Chapter 5.3) where possible. WHO's **AWaRe** list sorts antibiotics into Access (first choice), Watch (use carefully) and Reserve (last resort).
- **Infection prevention**: hand hygiene, sterilization and disinfection (Chapters 5.1 and 5.2), vaccination, clean water. Every infection prevented is a course of antibiotics not taken.
- **Surveillance and rules**: India's National Action Plan on AMR (2017) follows the WHO Global Action Plan (2015). Antibiotics in Schedule H1 must be sold only on prescription, and the 2016 Red Line campaign asks people not to buy medicines marked with a red vertical line without one.
- **Combination therapy**: in TB, several drugs are given together so that a mutant resistant to one is killed by another, as the worked example shows.

WORKED EXAMPLE

Question (6 marks). A TB patient's lung cavity holds 10^9 bacilli. Suppose the chance that a given cell carries a resistance mutation is 10^{-8} for drug A and 10^{-6} for drug B, independently. How many cells are expected to resist (a) drug A, (b) drug B, (c) both? What does this say about single-drug treatment?

1. (a) Expected A-resistant cells = $10^9 \times 10^{-8} = \mathbf{10}$.
2. (b) Expected B-resistant cells = $10^9 \times 10^{-6} = \mathbf{1000}$.
3. (c) Independent events multiply: chance of both = $10^{-8} \times 10^{-6} = 10^{-14}$. Expected doubly resistant cells = $10^9 \times 10^{-14} = \mathbf{10^{-5}}$, so almost certainly none.
4. With drug A alone, the 10 resistant cells survive and regrow into a fully resistant infection. With A and B together, each single mutant is killed by the other drug.

This is the reason TB is always treated with a combination of drugs, and why stopping one drug early or taking them irregularly breeds MDR-TB.

EXAM TRAPS

- Antibiotics select resistant mutants; they do not create the mutation. Write "selection", not "the bacteria get used to the drug".
- It is the bacterium that becomes resistant, not the patient.
- Persisters and biofilm cells are tolerant, not resistant: their offspring are still susceptible.
- MRSA resists through PBP2a (target change), not through a β-lactamase. Name the right mechanism.
- R plasmids move by conjugation; do not say "by transformation" unless the question is about free DNA.

CHECK YOURSELF

– REMEMBER **List the four main mechanisms of antibiotic resistance.** 2 marks

Enzymatic inactivation, target modification, reduced entry (permeability), and efflux pumps.

– REMEMBER **Expand ESKAPE.** 3 marks

Enterococcus faecium, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* species.

– UNDERSTAND **Distinguish intrinsic and acquired resistance with an example of each.** 4 marks

Intrinsic: natural to the species, e.g. *Mycoplasma* resists β-lactams because it has no cell wall. Acquired: gained by mutation or gene transfer in a once-susceptible species, e.g. MRSA gaining *mecA*.

– UNDERSTAND **Explain how MRSA resists methicillin.** 3 marks

The *mecA* gene encodes PBP2a, a transpeptidase with low affinity for β-lactams. It keeps cross-linking peptidoglycan when the normal PBPs are blocked, so the wall stays intact.

– APPLY **A population of 10^{10} cells has a resistance mutation frequency of 10^{-9}. How many resistant cells are expected?** 2 marks

$10^{10} \times 10^{-9} = 10$ resistant cells, enough to regrow under the drug.

– APPLY **A *Klebsiella* isolate destroys imipenem in a tube test. Which mechanism is this and why is it alarming?** 3 marks

Enzymatic inactivation by a carbapenemase (such as NDM-1 or KPC). Carbapenems are last-resort drugs, and carbapenemase genes travel on plasmids to other Gram-negatives.

– ANALYZE **Why does horizontal gene transfer make AMR spread faster than mutation alone?** 4 marks

Mutation must arise anew in each lineage and usually gives resistance to one drug. Plasmids, transposons and integrons carry ready-made genes for several drugs and move between cells and species, so a sensitive strain can become MDR in one transfer and resistance can cross from harmless gut bacteria to pathogens.

BEYOND THE SYLLABUS

Clinical labs now detect resistance genes directly with PCR panels and whole-genome sequencing instead of waiting for culture. Surveillance networks such as WHO GLASS and ICMR's AMR surveillance network pool such data, and wastewater sequencing tracks resistance genes in whole cities. Research looks at phage therapy, β-lactamase inhibitor combinations, anti-virulence drugs and new payment models to reward companies for drugs kept in reserve. Search "One Health AMR", "AWaRe antibiotic book" and "resistome" to read further. This is not for the exam.

5.5 Microbial spoilage of food and its control

Food spoils when microbes grow in it and change its smell, taste, texture or look until people refuse to eat it: sour milk, slimy chicken, mouldy bread, a bulging tin. Which microbe wins depends on the food itself (its water, acid and nutrients) and on how it is stored. Food preservation works by tilting those same factors against microbes, which is why this chapter uses every method from Chapters 5.1 and 5.2.

You need: D- and z-values from Chapter 5.1; growth and temperature classes (Unit 4, Bacterial growth curve and growth kinetics).

What decides who grows: intrinsic and extrinsic factors

Spoilage is not the same as food poisoning. A spoiled food may be harmless, and a food that looks and smells fine may carry *Salmonella*. Spoilage organisms announce themselves; many pathogens do not.

Intrinsic factors belong to the food:

- **Water activity** (a_W): the vapour pressure of water in the food divided by that of pure water at the same temperature. Pure water has $a_W = 1.00$. Salt and sugar bind water and lower a_W, and drying removes it. Figure 5.5.1 shows the approximate lowest a_W at which each group grows.
- **pH**: most bacteria prefer near-neutral foods. Foods above pH 4.6 (meat, fish, milk, most vegetables) are **low-acid foods** and are spoiled mainly by bacteria. Acid foods (most fruits, juices, pickles) are spoiled mainly by yeasts, moulds and acid-tolerant lactic acid bacteria. pH 4.6 matters because *Clostridium botulinum* does not grow or make toxin below it.
- **Redox potential** (Eh): aerobes spoil surfaces, anaerobes the inside of meat or a sealed can.
- **Nutrients, natural antimicrobials and structures**: protein-rich foods favour proteolytic bacteria; lysozyme in egg white, and compounds in garlic, cloves and turmeric, inhibit some microbes; shells, peels and skins keep microbes out until they are broken.

Extrinsic factors belong to storage: temperature (psychrotrophs such as *Pseudomonas* and the pathogen *Listeria monocytogenes* grow slowly in a fridge), relative humidity (damp storage lets moulds grow on dry foods), and the gas around the food (carbon dioxide slows many aerobes). **Implicit factors** are the interactions between microbes, such as lactic acid bacteria acidifying a food so others cannot grow.

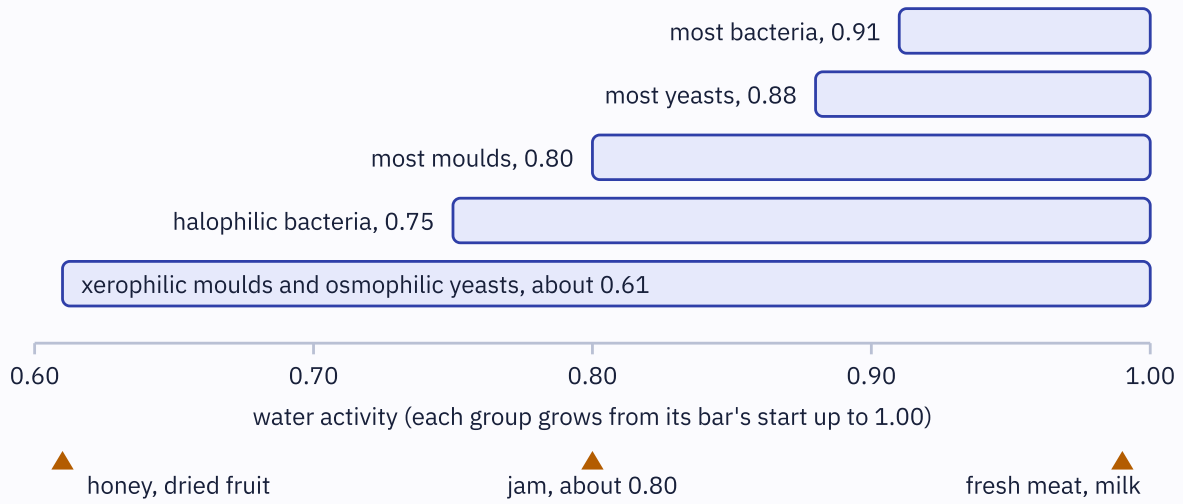


Figure 5.5.1 Approximate lowest water activity for growth of each group, with three foods marked. Notice that bacteria drop out first as a_w falls, so dry and sugary foods are spoiled by moulds and yeasts, not bacteria. The limits are typical values; individual species differ.

Who spoils what

FOOD	MAIN SPOILAGE ORGANISMS	WHAT YOU SEE
Raw milk	*Lactococcus lactis* and other lactic acid bacteria; in chilled milk, psychrotrophic *Pseudomonas*	souring and curdling; bitter or rancid taste from proteases and lipases
Chilled meat and poultry	*Pseudomonas* in air; lactic acid bacteria and *Brochothrix thermosphacta* in vacuum packs	surface slime and putrid smell; sour smell in vacuum packs
Fish	*Shewanella putrefaciens*, *Pseudomonas*	"fishy" trimethylamine from trimethylamine oxide (TMAO); hydrogen sulfide
Eggs	*Pseudomonas*, moulds, after shell defences fail	coloured rots, such as green rot
Fruits and vegetables	*Pectobacterium* (formerly *Erwinia*) on vegetables; moulds *Penicillium expansum*, *Botrytis cinerea*, *Rhizopus*	bacterial soft rot from pectinases; blue mould of apples, grey mould of berries
Bread	*Rhizopus stolonifer*, *Penicillium*, *Aspergillus*; *Bacillus subtilis* group	mould growth; "rope": sticky, stringy crumb with an odd smell
Canned low-acid food	*Geobacillus stearothermophilus*; *Thermoanaerobacterium thermosaccharolyticum*; *Desulfotomaculum nigrificans*	flat sour (acid, no gas); swollen can (gas); sulfide stinker (black, rotten-egg smell)

Canned-food spoilage by these thermophilic spore-formers usually means the cans were cooled slowly or stored hot. A swollen can of a low-acid food can also mean the process was too mild or the seam leaked, and it must be treated as a botulism risk.

Control: keep out, kill, or stop growth

1. **Keep out**: hygiene in handling, clean equipment, intact packaging.
2. **Kill**: blanching of vegetables, pasteurization of milk and juice, canning (below), irradiation, and high-pressure processing of juices and ready meals.
3. **Stop growth**: refrigeration at 5 °C or below, freezing at −18 °C, drying, salting and sugaring (lower a_W), acidifying or fermenting (lower pH), vacuum and modified-atmosphere packaging.
4. **Chemical preservatives** permitted by food law (FSSAI in India): benzoate in acid drinks and squashes, sorbate in cheese and bakery, propionate in bread against mould and rope, nitrite in cured meats against *C. botulinum*, sulfur dioxide in dried fruit and wine, and **nisin**, a bacteriocin made by *Lactococcus lactis*, in processed cheese.

Hurdle technology (Lothar Leistner) combines several mild barriers instead of one harsh one. A home-made lemon pickle is a good Indian example: lemon juice lowers pH, salt lowers a_W, a layer of oil keeps air out, and spices add antimicrobials. No single hurdle would hold, but together they keep the pickle safe for months.

Canning heats a sealed can to kill spores. For low-acid foods the target is the **botulinum cook**: a 12D reduction of *C. botulinum* spores. With D_{121} of about 0.2 minutes for these spores, 12D is about 2.5 minutes at 121 °C. The lethality of a real process, where the can's centre may be cooler, is added up as the **F_o value** using the **lethal rate** L = 10^((T − 121.1)/z), with z = 10 °C. L is how many minutes at 121.1 °C one minute at temperature T is worth.

WORKED EXAMPLE

Question (8 marks). The coldest point in a can of peas is held at 115 °C for 20 minutes (ignore heating and cooling). Take z = 10 °C. (a) Find the lethal rate and F_o. (b) Does it achieve a botulinum cook if D_{121} = 0.21 min? (c) How many log reductions does it give for flat-sour spores with D_{121} = 4 min?

1. (a) L = 10^((115 − 121.1)/10) = 10^(−0.61) = 0.245. So one minute at 115 °C equals 0.245 min at 121.1 °C.
2. F_o = L × time = 0.245 × 20 = **4.9 min**.
3. (b) 12D for *C. botulinum* = 12 × 0.21 = 2.52 min. Since 4.9 > 2.52, yes. It gives 4.9 ÷ 0.21 = **about 23 log reductions**.
4. (c) For flat-sour spores: 4.9 ÷ 4 = **about 1.2 log reductions**, so many survive.
5. Conclusion: the can is safe from botulism but can still go flat sour. The surviving thermophiles grow only if the can is cooled slowly or stored warm (they grow best around 50 to 60 °C), so rapid cooling and cool storage are the control.

EXAM TRAPS

- Water activity is not water content. Jam has plenty of water, but sugar binds it, so its a_w is low.
- Refrigeration slows microbes; it does not kill them, and psychrotrophs keep growing.
- The 4.6 pH line is about *C. botulinum*, not about all bacteria.
- Flat sour means acid without gas, so the can looks normal. Do not describe it as a swollen can.
- F_o is in minutes at 121.1 °C, not in log reductions. Divide by D to get logs.

CHECK YOURSELF

– REMEMBER **Define water activity and give its value for pure water.** 2 marks

The ratio of the water vapour pressure of a food to that of pure water at the same temperature; pure water has a_w = 1.00.

– REMEMBER **Name the organism that causes rope in bread and one preservative used against it.** 2 marks

Bacillus subtilis (and related *Bacillus* species); calcium or sodium propionate.

– UNDERSTAND **Why are fruits spoiled mainly by moulds and yeasts but meat mainly by bacteria?** 3 marks

Fruits are acidic (low pH) and often sugary, conditions that moulds and yeasts tolerate better than most bacteria. Meat is near neutral, high in water and protein, which favours fast-growing bacteria that outcompete fungi.

– UNDERSTAND **Explain hurdle technology with an example.** 4 marks

Several mild preservation factors are combined so that together they stop microbes while each alone would not, and food quality suffers less. Lemon pickle: low pH, low a_w from salt, oil excluding air, and spices.

– APPLY **Find the lethal rate at 111.1 °C (z = 10 °C) and the F_o of holding 30 min there.** 3 marks

L = 10^((111.1 – 121.1)/10) = 10^{-1} = 0.1; F_o = 0.1 × 30 = 3 min.

– APPLY **A sugar syrup has a_w 0.85. Using Figure 5.5.1, which groups can grow in it?** 2 marks

Most moulds (to about 0.80), halophiles in principle, and xerophilic moulds and osmophilic yeasts; most bacteria (0.91) and most yeasts (0.88) cannot.

– ANALYZE **Canned peas that passed a botulinum cook turned sour in a hot godown in May. Explain and suggest a fix.** 4 marks

A botulinum cook does not kill highly heat-resistant thermophilic spores such as *Geobacillus stearothermophilus*. Hot storage let them germinate and make acid without gas (flat sour). Fix: cool cans quickly after processing, store them cool, and reduce spore load in raw materials such as sugar and starch.

BEYOND THE SYLLABUS

Food companies now use predictive microbiology, models that forecast growth from temperature, pH and a_w, to set shelf life without testing every batch. Food safety systems such as HACCP place control points at the steps where spoilage and pathogens are stopped. Newer non-thermal methods include high-pressure processing, pulsed electric fields and cold plasma, and smart packaging with time–temperature indicators. Search "predictive microbiology ComBase" and "HACCP plan" to read further. This is not for the exam.

Unit summary

TERM	MEANING OR FORMULA	CHAPTER
Sterilization	removal or killing of all life, including endospores	5.1
Survivor equation	$N = N_0 \times 10^{(-t/D)}$	5.1
D-value	time at a stated temperature for a 90% (one log) kill	5.1
z-value	temperature rise that cuts D tenfold; $D_T = D_{ref} \times 10^{((T_{ref} - T)/z)}$	5.1
Autoclave	saturated steam, 121 °C, about 15 psi above atmospheric; 15 min small loads, longer for wrapped loads	5.1
Hot air oven	160 °C for 2 h or 170 °C for 1 h	5.1
Watson's law	$C^n \times t = K$; n is the concentration exponent (about 6 for phenol)	5.2
Phenol coefficient	dilution of agent ÷ dilution of phenol giving the same kill	5.2
MIC / MBC	lowest concentration that inhibits visible growth / kills 99.9%	5.3
Bactericidal rule	MBC ÷ MIC ≤ 4	5.3
Resistance mechanisms	inactivation, target change, reduced entry, efflux (and bypass)	5.4
Expected mutants	population × mutation frequency; for two drugs multiply the frequencies	5.4
Water activity	a_w = vapour pressure of food water ÷ that of pure water	5.5
Lethal rate and F_0	$L = 10^{((T - 121.1)/z)}$; $F_0 = \Sigma L \times \Delta t$, in minutes at 121.1 °C	5.5
Botulinum cook	12D for *C. botulinum* spores in low-acid foods (pH above 4.6)	5.5

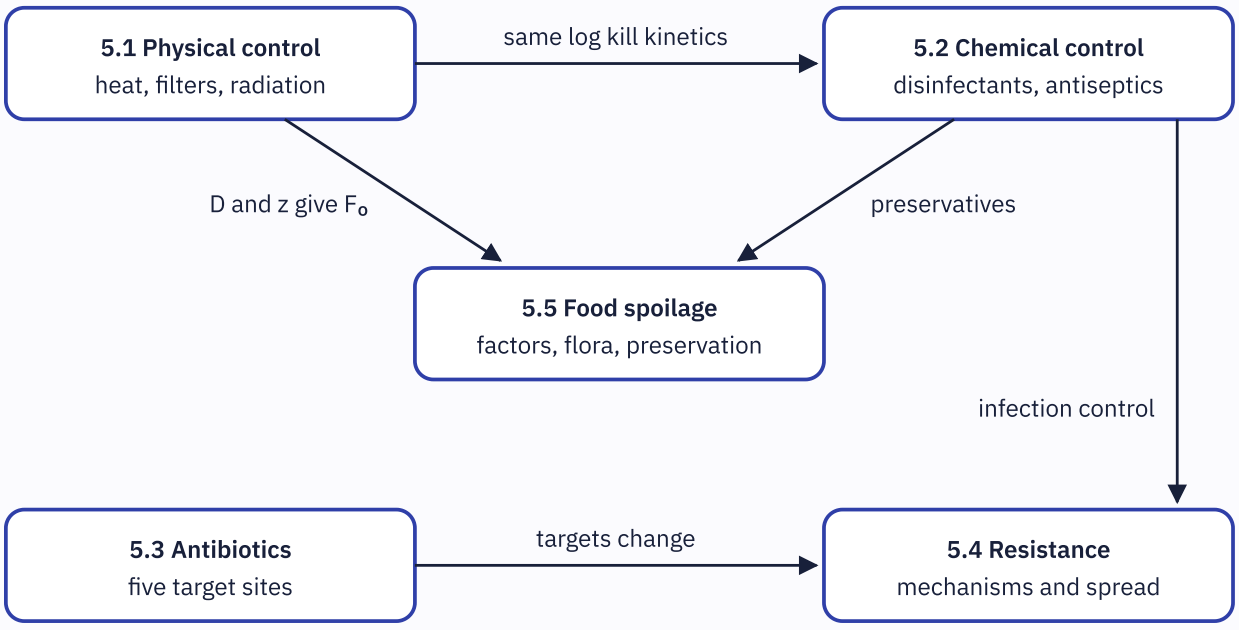


Figure 5.S.1 How the five chapters connect. The top row works outside the body, the bottom row inside it, and food preservation draws on both physical and chemical methods.

1. Sterilization kills everything including spores; disinfection, antisepsis and sanitization only lower numbers.
2. Microbes die logarithmically: each D minutes kills 90%, so a sterile result is a probability (SAL 10^{-6}), never a certainty.
3. Moist heat beats dry heat; the autoclave at 121 °C is the standard, the hot air oven is for glass, oils and powders.
4. Filtration removes microbes from heat-sensitive liquids and air; gamma rays sterilize packed plastics; UV only treats surfaces.
5. Pick a chemical agent by the hardest microbe it must kill and the material; clean first, use the right dilution and contact time.
6. Antibiotics hit five targets: wall, membrane, ribosome, nucleic acid synthesis and folate synthesis; selective toxicity comes from differences between bacterial and human cells.
7. Bacteria resist by destroying the drug, changing the target, keeping the drug out or pumping it out, and they share resistance genes on plasmids.
8. Food spoilage depends on a_W, pH, Eh, nutrients and storage; preservation combines hurdles, and canning low-acid food needs a 12D botulinum cook.

Exam question bank

Remember and understand

- REMEMBER **Describe the construction, working and uses of an autoclave, and how its performance is checked.** 10 marks

  Pressure vessel, steam supply, air exhaust, safety valve and gauge; air removed, steam at about 15 psi gives 121 °C; times (15 min small loads, longer wrapped); uses (media, instruments, waste); checks: chemical tape, biological indicator *G. stearothermophilus*; common failures (trapped air, overloading).

- UNDERSTAND **Classify chemical disinfectants with one example, mode of action and use for each class.** 12 marks

  Phenolics, alcohols, halogens, biguanides, quaternary ammonium compounds, heavy metals, aldehydes, ethylene oxide, peroxygens; for each: example, target (membrane, protein, nucleic acid), use and limitation; mention the resistance ladder.

- UNDERSTAND **Explain the mechanism of action of antibiotics that inhibit cell wall and protein synthesis.** 10 marks

  β-lactams bind PBPs and block cross-linking; vancomycin binds D-Ala-D-Ala; 70S vs 80S ribosomes; aminoglycosides and tetracyclines on 30S; macrolides, chloramphenicol and linezolid on 50S; bactericidal vs bacteriostatic; a labelled diagram.

- UNDERSTAND **Discuss the intrinsic and extrinsic factors that affect microbial spoilage of food.** 10 marks

  a_w with group limits, pH and the 4.6 line, Eh, nutrients, natural antimicrobials, structures; temperature and psychrotrophs, humidity, gas atmosphere; implicit factors; one example food for each.

Apply

- APPLY **A medium contains 10^6 spores per mL in 500 mL; D_{121} = 2 min, z = 10 °C. Find the time to SAL 10^{-6} at 121 °C and at 111 °C.** 8 marks

  $N_o = 5 \times 10^8$, log 8.7; reductions to −6: 14.7; at 121 °C: 14.7 × 2 = 29.4 min; D_{111} = 20 min, so 294 min. State the assumption that the load is at temperature throughout.

– APPLY **Plan the sterilization and disinfection of every item in a microbiology teaching lab, with reasons.** 12 marks

Media and discarded cultures: autoclave; glass pipettes and Petri dishes: hot air oven; antibiotic and vitamin stocks: 0.22 µm filtration; plastic plates: bought gamma-irradiated; bench: 70% alcohol or phenolic; spills: hypochlorite after removing organic load; air: HEPA in the hood, UV when empty; loops: flaming. Spans Chapters 5.1 and 5.2.

– APPLY **Given broth dilution results, find MIC and MBC and interpret them; explain how Kirby–Bauer results are read.** 8 marks

MIC as first clear tube, MBC from subculture, MBC ÷ MIC ≤ 4 means bactericidal; disk diffusion on Mueller–Hinton agar, zone diameter compared with CLSI or EUCAST breakpoints, S/I/R, no cross-drug comparison.

– APPLY **A canning process holds the cold point at 118 °C for 12 min. With z = 10 °C, find F_o and judge it against a botulinum cook (D_{121} = 0.21 min).** 8 marks

L = 10^((118 – 121.1)/10) = 10^(−0.31) = 0.49; F_o = 0.49 × 12 = 5.9 min; 12D = 2.52 min, so it passes, with about 28 log reductions.

Analyze and evaluate

– ANALYZE **Compare physical and chemical methods of microbial control and argue which suits a hospital operating theatre.** 12 marks

Table of methods with what each kills, materials, speed, residues and cost; Spaulding classes; theatre: instruments autoclaved, heat-sensitive devices with EtO or H_2O_2 plasma, HEPA air, surfaces with chemical disinfectants, skin with antiseptics. Spans 5.1 and 5.2.

– ANALYZE **For each antibiotic target, name a drug class and a resistance mechanism that defeats it.** 12 marks

Wall: β-lactams vs β-lactamases and PBP2a; vancomycin vs D-Ala-D-Lac. Ribosome: aminoglycosides vs modifying enzymes; tetracyclines vs efflux; macrolides vs *erm* methylation. DNA: quinolones vs gyrase mutations; rifampicin vs *rpoB*. Folate: sulfonamides and trimethoprim vs *sul* and *dfr* bypass. Spans 5.3 and 5.4.

– EVALUATE **"AMR is a One Health problem." Evaluate this statement and the measures India has taken.** 16 marks

Shared bacteria and genes across humans, animals and environment; drivers (self-medication, growth promoters, hospital spread, effluent); horizontal gene transfer; GBD 2019 figures; measures: National Action Plan 2017, Schedule H1, Red Line campaign, AWaRe, surveillance, infection control; gaps and what else is needed.

– ANALYZE **Explain why TB is treated with drug combinations, using mutation frequencies.** 8 marks

Large bacillary load; single-drug resistant mutants exist before treatment; independent frequencies multiply; worked numbers; consequences of irregular treatment (MDR-TB).

– ANALYZE **Explain the types of spoilage of canned foods and how each points to a cause in processing or storage.** 10 marks

Flat sour (thermophilic *Geobacillus*, warm storage), thermophilic swell, sulfide stinker; mesophilic spoilage and swells pointing to under-processing or leaking seams; botulism risk; F_0 and 12D reasoning; controls. Spans 5.1 and 5.5.

Glossary

Antibiotic	A microbial product, or its derivative, that inhibits or kills other microbes at low concentration.
Antiseptic	A chemical mild enough to reduce microbes on living tissue.
Autoclave	Pressure vessel that sterilizes with saturated steam, usually at 121 °C.
AWaRe	WHO grouping of antibiotics into Access, Watch and Reserve.
Bactericidal / bacteriostatic	Kills bacteria / only stops their growth.
β-lactamase	Bacterial enzyme that breaks the β-lactam ring of penicillins and related drugs.
Biological indicator	Resistant spores used to prove a sterilization cycle worked.
Carbapenemase	β-lactamase that destroys carbapenems, such as NDM-1 or KPC.
D-value	Time at a given temperature to kill 90% of a population.
Disinfectant	A chemical that kills most pathogens on non-living objects.
Efflux pump	Membrane protein that pumps a drug out of the cell.

F_0 value	Lethality of a heat process in equivalent minutes at 121.1 °C (z = 10 °C).
Flat sour	Canned-food spoilage with acid but no gas, by thermophilic spore-formers.
HEPA filter	Air filter that holds at least 99.97% of 0.3 µm particles.
Hurdle technology	Food preservation by combining several mild barriers to growth.
MIC	Minimum inhibitory concentration: lowest drug level that stops visible growth.
MRSA	*Staphylococcus aureus* resistant to methicillin, usually through *mecA* and PBP2a.
Pasteurization	Mild heat (63 °C for 30 min or 72 °C for 15 s) that kills pathogens in milk without sterilizing it.
Penicillin-binding protein	Transpeptidase that cross-links peptidoglycan; the target of β-lactams.
Persister	Dormant cell that survives a drug without being genetically resistant.
Phenol coefficient	Effectiveness of a disinfectant compared with phenol in a standard test.
R plasmid	Plasmid carrying antibiotic resistance genes, often transferable by conjugation.
Selective toxicity	Harming the microbe more than the host.
Sterility assurance level	Probability that an item still carries a live microbe after sterilization, often 10^{-6}.
Sterilization	Destruction or removal of all life, including endospores.
Tyndallization	Steaming at 100 °C on three successive days to kill spores after they germinate.
Water activity	Ratio of water vapour pressure of a food to that of pure water; available water.
z-value	Temperature change that alters the D-value tenfold.

Sources and further reading

Textbooks

- Willey, Sandman and Wood, *Prescott's Microbiology*, McGraw-Hill: chapters on control of microorganisms, antimicrobial chemotherapy and food microbiology (from memory; check).

- Pelczar, Chan and Krieg, *Microbiology*, McGraw-Hill (from memory; check).
- Tortora, Funke and Case, *Microbiology: An Introduction*, Pearson (from memory; check).
- Ananthanarayan and Paniker's *Textbook of Microbiology*, Universities Press: sterilization, disinfection and antibiotic chapters (from memory; check).
- Jay, Loessner and Golden, *Modern Food Microbiology*, Springer: intrinsic and extrinsic factors and the water activity limits in Figure 5.5.1 (from memory; check).
- Frazier and Westhoff, *Food Microbiology*, McGraw-Hill: spoilage by food group (from memory; check).

Open web resources

- US CDC, Guideline for Disinfection and Sterilization in Healthcare Facilities (2008), Table 7: minimum cycle times for steam sterilization, cdc.gov. Source of the 30 min (121 °C, wrapped, gravity) and 4 min (132 °C, prevacuum) figures.
- Antimicrobial Resistance Collaborators, "Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis", *The Lancet* 2022;399:629–655, open text at PubMed Central. Source of the 1.27 million and 4.95 million figures.

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