Microbiology

Unit 5 · Lesson 2 · Antibiotics, resistance and food spoilage

The notes for Lesson 2 of Unit 5: chapters 5.3 to 5.5 of the unit book, with their figures, worked examples, exam traps and quizzes, in one file. The full unit book, with every chapter, the exam question bank and the glossary, is linked at the end.

Lesson 2 of 2 | 3 chapters | Chapters 5.3 to 5.5

About these notes

These are the notes for **Lesson 2 · Antibiotics, resistance and food spoilage** of the University free n easy video lessons on Microbiology Unit 5, Control of Microorganisms. They are chapters 5.3 to 5.5 of the unit book, unchanged, so a page here is the same page in the full book.

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

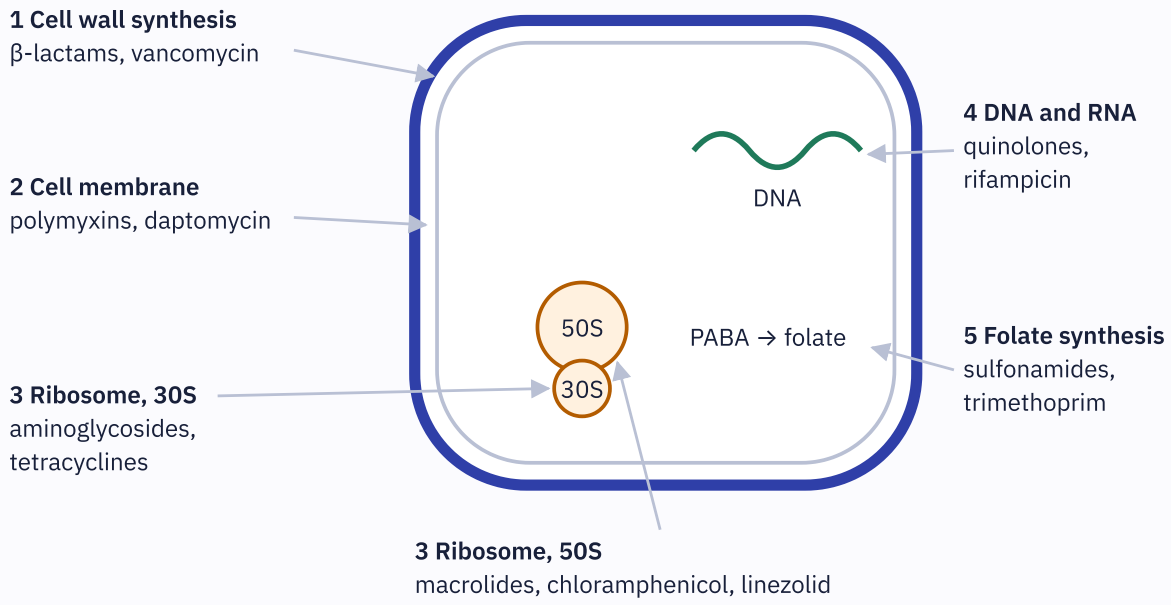


Figure 5.3.1 Where the main antibiotic classes act on a bacterial cell. Each target is either absent from human cells (peptidoglycan, the folate-making enzymes) or built differently there (80S ribosomes, human topoisomerases). Notice that one ribosome holds two targets: the 30S and the 50S subunits.

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

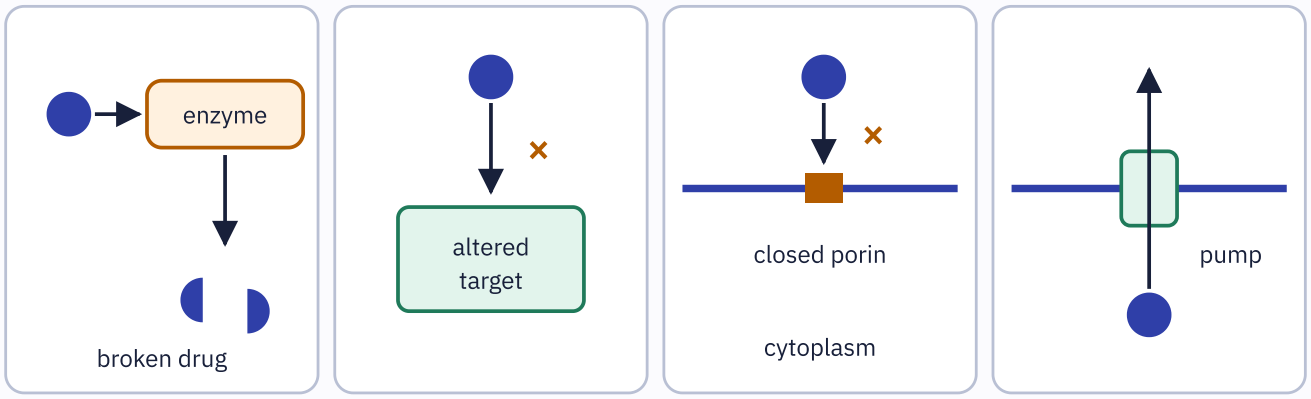


Figure 5.4.1 The four main resistance mechanisms; the blue circle is the drug. Panels 3 and 4 show the cell envelope as a blue line with the outside above it. Notice that only mechanism 1 destroys the drug; the other three keep it from reaching or binding its target.

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.

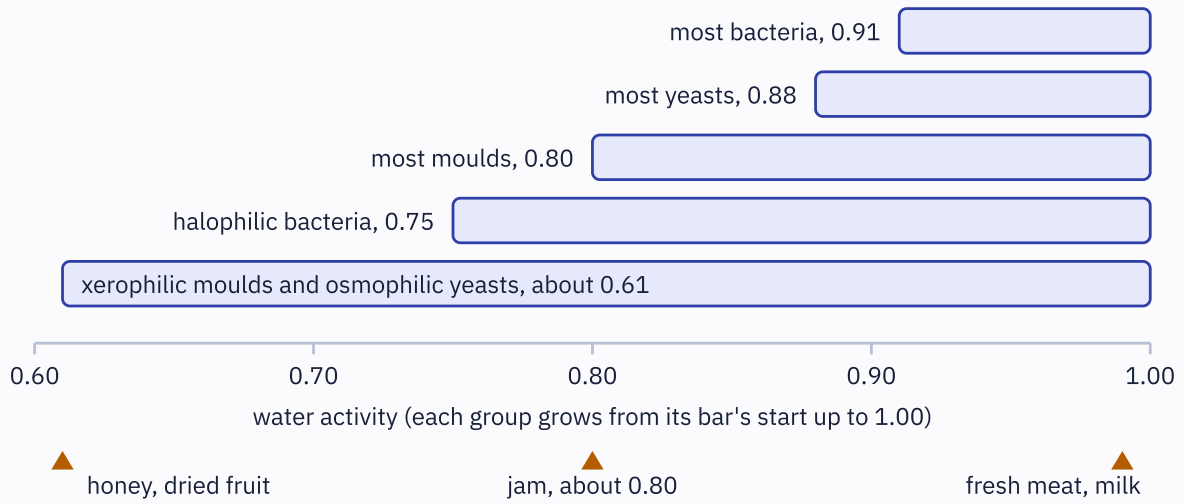


Figure 5.5.1 Approximate lowest water activity for growth of each group, with three foods marked. Notice that bacteria drop out first as a_w falls, so dry and sugary foods are spoiled by moulds and yeasts, not bacteria. The limits are typical values; individual species differ.

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_0 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.

Key terms of this lesson

TERM	MEANING OR FORMULA	CHAPTER
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_o	L = 10^((T − 121.1)/z); F_o = Σ L × Δt, 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

Glossary (terms used in this lesson)

Antibiotic A microbial product, or its derivative, that inhibits or kills other microbes at low concentration.

AWaRe WHO grouping of antibiotics into Access, Watch and Reserve.

β-lactamase Bacterial enzyme that breaks the β-lactam ring of penicillins and related drugs.

Carbapenemase β-lactamase that destroys carbapenems, such as NDM-1 or KPC.

Disinfectant A chemical that kills most pathogens on non-living objects.

Efflux pump Membrane protein that pumps a drug out of the cell.

F_o 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.

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.
R plasmid	Plasmid carrying antibiotic resistance genes, often transferable by conjugation.
Selective toxicity	Harming the microbe more than the host.
Sterilization	Destruction or removal of all life, including endospores.
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.

The full unit book

Every chapter of Unit 5, the unit summary, the exam question bank with model answers and the full glossary: Microbiology · Unit 5 · Control of Microorganisms (one file, works offline).

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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