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HIGHLIGHTED HANDOUT

MIC501T

Microbiology (Theory)

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HANDOUT

This course covers the fundamentals of microbiology: microbial cell structure and function, growth and control, metabolism, genetics, immunity, major pathogen groups, and applied microbiology in food, industry, and the environment.

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MODULE 1: INTRODUCTION TO MICROBIOLOGY AND THE MICROBIAL WORLD

Learning Objectives

- Define microbiology and its major branches
- Summarize the history from Leeuwenhoek to the germ theory
- Classify microorganisms and compare cell types
- Explain why microbes matter to humans

Scope of Microbiology

Microbiology is the study of organisms too small to be seen with the naked eye — bacteria, archaea, fungi, protozoa, algae, and viruses. Branches: **bacteriology**, **virology**, **mycology** (fungi), **parasitology**, **immunology**; applied branches include **medical, food, industrial, and environmental** microbiology. Microbes drive nutrient cycles, cause disease, produce food and drugs, and outnumber human cells in our own bodies.

History in Brief

Leeuwenhoek (1670s) first observed 'animalcules' with simple microscopes. **Pasteur** disproved spontaneous generation (swan-neck flask), founded germ theory, and created pasteurization and vaccines. **Koch** formulated **Koch's postulates** linking specific microbes to specific diseases and developed pure-culture techniques. **Fleming** discovered penicillin (1928); **Jenner** pioneered smallpox vaccination. The microscope, pure culture, and molecular methods are the field's three revolutions.

Cell Types Compared

Prokaryotes (bacteria, archaea): no membrane-bound nucleus, 70S ribosomes, usually single circular chromosome. **Eukaryotes** (fungi, protozoa, algae): true nucleus, 80S ribosomes, organelles. **Viruses**: acellular — nucleic acid in a protein coat, obligate intracellular parasites. **Archaea** resemble bacteria structurally but are genetically closer to eukaryotes and thrive in extreme environments. Size ladder: viruses (nm) < bacteria (µm) < eukaryotic microbes.

Key Terms & Definitions

Koch's postulates	Four criteria proving a microbe causes a disease.
Germ theory	Microorganisms cause infectious disease.
Acellular	Not composed of cells (viruses).
Normal flora	Microbes normally inhabiting the human body.

Example: Pasteur's Swan-Neck Flask

Pasteur boiled broth in flasks with S-shaped necks: air entered, but dust settled in the bend — broth stayed sterile for months. Tipping the flask washed dust in, and microbes grew. Spontaneous generation was dead; every microbe comes from a microbe — the basis of all aseptic technique.

Module Summary

- Microbiology studies bacteria, archaea, fungi, protozoa, algae, viruses.
- History: Leeuwenhoek → Pasteur → Koch → Fleming; three method revolutions.
- Prokaryote vs. eukaryote vs. acellular virus.
- Microbes: pathogens, but also nutrient cyclers, food/drug producers.

Exam Pointers (Very Important)

- ★ State and explain Koch's postulates.
- ★ How did Pasteur disprove spontaneous generation?
- ★ Compare prokaryotic, eukaryotic, and viral structure.
- ★ Name five branches of microbiology.

MODULE 2: BACTERIAL CELL STRUCTURE AND FUNCTION

Learning Objectives

- Draw and label the bacterial cell
- Explain the Gram stain mechanism
- Compare Gram-positive and Gram-negative walls
- Describe motility, capsules, and endospores

The Cell Envelope

From outside in: **glycocalyx** (capsule/slime layer — virulence, biofilms) → **cell wall** → **cell membrane**. **Gram-positive** walls: thick **peptidoglycan** (many layers) + teichoic acids; retain crystal violet (purple). **Gram-negative**: thin peptidoglycan + **outer membrane** containing **LPS/endotoxin**; lose crystal violet, counterstain pink with safranin. **Acid-fast** bacteria (Mycobacterium) have waxy **mycolic acids** — resist decolorization.

Internal Structures

Nucleoid: circular chromosome (no histones) + **plasmids** (extra-chromosomal DNA carrying antibiotic resistance, toxins). **Ribosomes (70S)**: protein synthesis — target of many antibiotics. **Inclusions**: storage granules (glycogen, PHB). Some bacteria form **endospores** (Bacillus, Clostridium) — dormant, heat/chemical-resistant survival forms; **sterilization must kill spores**, disinfection need not.

Motility and Appendages

Flagella: rotating filaments for swimming (run-and-tumble chemotaxis toward nutrients). Arrangements: monotrichous, lophotrichous, amphitrichous, peritrichous. **Pili/fimbriae**: attachment; **sex pili** mediate conjugation. Biofilms — surface-attached communities in a polysaccharide matrix — resist antibiotics and disinfectants and cause chronic infections and industrial fouling.

Key Terms & Definitions

Peptidoglycan	Mesh of sugars + amino acids giving the wall strength.
Endotoxin (LPS)	Gram-negative outer-membrane toxin causing fever/shock.
Endospore	Dormant, highly resistant bacterial survival form.
Plasmid	Small circular DNA carrying extra genes (e.g., resistance).

Example: Why the Gram Stain Rules the Lab

A sputum Gram stain shows Gram-positive diplococci (pairs) — the clinician starts penicillin for likely pneumococcus within an hour, days before culture results. One stain narrows the entire antibiotic choice: purple vs. pink decides therapy.

Module Summary

- Envelope: glycocalyx → wall → membrane; Gram+ thick peptidoglycan, Gram– thin + LPS outer membrane.
- Plasmids carry resistance; 70S ribosomes are antibiotic targets.
- Endospores (Bacillus, Clostridium) survive sterilization-level insults.
- Flagella, pili, biofilms mediate movement, attachment, persistence.

Exam Pointers (Very Important)

- ★ Explain the mechanism of the Gram stain step by step.
- ★ Compare Gram-positive and Gram-negative cell walls.
- ★ What is an endospore and why does it matter for sterilization?
- ★ Distinguish pili from flagella.

MODULE 3: MICROBIAL GROWTH AND ITS CONTROL

Learning Objectives

- Describe the bacterial growth curve
- Explain physical and chemical growth requirements
- Compare sterilization, disinfection, and antisepsis
- Select appropriate control methods

Growth Requirements and Curve

Requirements: carbon/energy source, nitrogen, minerals, water; **oxygen classes** — obligate aerobes, anaerobes, facultative anaerobes, microaerophiles; **pH** (most neutrophiles), **temperature classes** — psychrophiles, mesophiles (most pathogens, 37°C), thermophiles; osmotic pressure (halophiles). Growth curve phases: **lag** (adaptation), **log/exponential** (binary fission, generation time), **stationary** (nutrient depletion, secondary metabolites/antibiotics made here), **death** phase. Generation time: E. coli ~20 min — one cell → millions overnight.

Physical Control Methods

Sterilization destroys ALL microbes including spores; **disinfection** kills pathogens on inanimate objects; **antisepsis** on living tissue; **sanitization** reduces to safe levels. Heat: **autoclave** (121°C, 15 psi, 15 min — moist heat, kills spores) is the gold standard; dry heat (hot-air oven) for glassware; **pasteurization** reduces (not sterilizes) milk; filtration sterilizes heat-labile liquids (0.22 µm); radiation (UV, gamma) for surfaces/disposables.

Chemical Agents and Evaluation

Phenolics, halogens (chlorine, iodine), alcohols (60-90% ethanol), heavy metals, quaternary ammonium compounds, aldehydes (glutaraldehyde — sporicidal), gases (ethylene oxide). Effectiveness depends on **concentration, contact time, organic load, pH, temperature**. Evaluated by **phenol coefficient** and use-dilution tests. Note: **antibiotics are for therapy inside the body; disinfectants are too toxic for tissues** — different tools, different rules.

Key Terms & Definitions

Generation time	Time for a bacterial population to double.
Autoclave	Steam under pressure: 121°C, 15 psi, 15 min.
Bactericidal vs. bacteriostatic	Kills bacteria vs. inhibits their growth.
Decimal reduction time (D-value)	Time to kill 90% of a population at a given temperature.

Example: Why Boiling Isn't Sterilizing

Boiling (100°C) kills vegetative cells but not endospores — *Clostridium tetani* spores survive. That's why surgical instruments are autoclaved (121°C pressurized steam), not boiled. Temperature alone doesn't sterilize; temperature + pressure + time does.

Module Summary

- Growth needs: nutrients, O₂ class, pH, temperature class, water.
- Curve: lag → log → stationary (antibiotics made here) → death.
- Sterilization > disinfection > antisepsis > sanitization.
- Autoclave is the gold standard; chemicals judged by concentration, time, organic load.

Exam Pointers (Very Important)

- ★ Draw and label the bacterial growth curve, explaining each phase.
- ★ Distinguish sterilization, disinfection, antisepsis, sanitization.
- ★ Why does the autoclave kill spores but boiling does not?
- ★ Classify bacteria by oxygen and temperature requirements.

MODULE 4: MICROBIAL METABOLISM

Learning Objectives

- Summarize energy generation: respiration vs. fermentation
- Compare metabolic diversity (photo/auto/heterotrophs)
- Explain key pathways: glycolysis, Krebs, ETC
- Link metabolism to industrial applications

Energy and Carbon Sources

Classification by energy source: **phototrophs** (light) vs. **chemotrophs** (chemicals); by carbon source: **autotrophs** (CO₂) vs. **heterotrophs** (organic carbon). Combinations: photoautotrophs (cyanobacteria, plants), chemoheterotrophs (most bacteria, all pathogens — eat organic matter), chemolithotrophs (oxidize inorganic compounds — nitrifiers). This diversity lets microbes live everywhere from sulfur vents to the human gut.

Respiration vs. Fermentation

Aerobic respiration: glycolysis → Krebs cycle → **electron transport chain** with O₂ as final acceptor — ~38 ATP per glucose, complete oxidation to CO₂. **Anaerobic respiration:** same chain, different final acceptor (nitrate, sulfate). **Fermentation:** no ETC — pyruvate reduced to regenerate NAD⁺; only ~2 ATP; products define the type — **lactic acid** (Lactobacillus), **alcoholic** (yeast), mixed-acid, butyric. Fermentation products are the basis of food microbiology and industry.

Enzymes and Regulation

Microbial enzymes: **exoenzymes** digest macromolecules outside the cell (amylases, proteases — used in food tests and industry); **endoenzymes** work inside. Regulation: **feedback inhibition** and **enzyme induction/repression** (lac operon — genes switched on only when lactose is present). Understanding regulation lets industry **overproduce** metabolites (amino acids, antibiotics) by deregulating pathways.

Key Terms & Definitions

Chemolithotroph	Gains energy by oxidizing inorganic compounds.
Fermentation	ATP generation without an electron transport chain (~2 ATP).
Exoenzyme	Secreted enzyme digesting substrates outside the cell.
Operon	Cluster of coordinately regulated bacterial genes (e.g., lac).

Example: From Pathway to Product

Yeast fermenting glucose → ethanol + CO₂ is alcoholic fermentation — the same reaction behind bread (CO₂ leavens) and beer (ethanol). Industry scales one microbial pathway into billion-dollar food and fuel sectors.

Module Summary

- Classify by energy (photo/chemo) × carbon (auto/hetero) source.
- Aerobic respiration ~38 ATP; fermentation ~2 ATP with characteristic products.
- Exoenzymes digest outside; operons regulate pathways.
- Metabolic diversity = industrial opportunity.

Exam Pointers (Very Important)

- ★ Compare aerobic respiration, anaerobic respiration, and fermentation.
- ★ Classify microbes by energy and carbon source with examples.
- ★ What is an operon? Explain the lac operon.
- ★ Why is fermentation industrially important?

MODULE 5: MICROBIAL GENETICS

Learning Objectives

- Describe bacterial DNA replication, transcription, translation
- Explain mutation and its consequences
- Compare transformation, transduction, conjugation
- Explain how antibiotic resistance spreads

Information Flow

Central dogma: DNA → RNA → protein. Bacterial replication is **semi-conservative**, starting at **oriC**; transcription by **RNA polymerase** (no nucleus — transcription and translation are **coupled**); translation on **70S ribosomes**. Differences from eukaryotes (coupled transcription-translation, no introns, operons) are **antibiotic targets**: rifampin hits RNA polymerase; tetracyclines/aminoglycosides hit 70S ribosomes — selective toxicity from molecular differences.

Mutation

Mutations: **point** (substitution — silent, missense, nonsense), **frameshift** (insertion/deletion), spontaneous (replication errors) vs. **induced** (UV, chemicals — Ames test detects mutagens). Most mutations are neutral or harmful, but rare beneficial ones (antibiotic resistance, new metabolic abilities) drive **evolution**. **Mutants** are screened for desired traits in strain improvement for industry.

Gene Transfer — The Resistance Highway

Three mechanisms: **transformation** (uptake of naked DNA — Griffith's pneumococcus experiment), **transduction** (bacteriophage-mediated), **conjugation** (F-plasmid, cell-to-cell via sex pilus) — the main route for **R-plasmids** carrying multiple antibiotic resistances. **Transposons** ('jumping genes') move resistance between plasmid and chromosome. Consequence: resistance spreads **horizontally** between species — the reason antibiotic stewardship is a global emergency.

Key Terms & Definitions

Conjugation	Plasmid transfer through direct cell contact.
R-plasmid	Plasmid carrying antibiotic-resistance genes.
Transposon	Mobile DNA segment that relocates within/between genomes.
Selective toxicity	Harming microbes without harming the host.

Example: One Plasmid, Five Resistances

A single conjugative R-plasmid can carry resistance to penicillin, tetracycline, chloramphenicol, sulfonamides, and streptomycin — transferred in minutes to a neighboring *E. coli* in the gut. One conjugation event can create a multidrug-resistant pathogen: this is why incomplete antibiotic courses are dangerous.

Module Summary

- Central dogma with bacterial twists (coupled, operons) = drug targets.
- Mutations: point, frameshift; spontaneous vs. induced.
- Gene transfer: transformation, transduction, conjugation (+ transposons).
- Horizontal transfer of R-plasmids drives the resistance crisis.

Exam Pointers (Very Important)

- ★ Compare transformation, transduction, and conjugation.
- ★ How do R-plasmids spread antibiotic resistance?
- ★ Why are bacterial ribosomes good antibiotic targets?
- ★ Distinguish spontaneous from induced mutation.

MODULE 6: IMMUNITY AND HOST DEFENSE

Learning Objectives

- Compare innate and adaptive immunity
- Explain antibody structure and function
- Describe cell-mediated immunity
- Explain vaccines and hypersensitivity

Innate Defenses

First line: skin, mucous membranes, stomach acid, normal flora competition. **Second line:** **phagocytosis** (neutrophils, macrophages), **inflammation** (redness, heat, swelling, pain — delivers defenders), **fever** (inhibits microbes), **interferons** (antiviral), **complement** (protein cascade: opsonization, chemotaxis, lysis via MAC). Innate = fast, non-specific, no memory.

Adaptive Immunity

Humoral (B cells → plasma cells → **antibodies**): antibody = Y-shaped, two heavy + two light chains; functions — neutralization, opsonization, complement activation, ADCC. Classes: **IgG** (blood, crosses placenta), **IgA** (mucosal secretions), **IgM** (first response, pentamer), **IgE** (allergy/parasites), **IgD**. **Cell-mediated** (T cells): cytotoxic T cells kill infected cells; helper T cells coordinate. **Memory cells** make secondary responses faster and stronger — the basis of vaccination.

Vaccines and Hypersensitivity

Vaccines: **live attenuated** (strong, e.g., BCG, OPV), **inactivated/killed**, **toxoid** (diphtheria, tetanus), **subunit/recombinant** (hepatitis B). **Herd immunity** protects the unvaccinated when coverage is high. **Hypersensitivity:** Type I (IgE anaphylaxis), II (cytotoxic), III (immune complex), IV (delayed, T-cell). Serology (agglutination, ELISA) exploits antibody specificity for diagnosis.

Key Terms & Definitions

Opsonization	Coating microbes to enhance phagocytosis.
Herd immunity	Community protection from high vaccination coverage.
Attenuated vaccine	Weakened live pathogen vaccine.
Complement	Plasma protein cascade aiding pathogen destruction.

Example: Why Boosters Work

The first tetanus shot builds memory cells slowly; the booster triggers them — antibody titers soar 10-100x within days. Vaccination doesn't just protect the individual: with herd immunity, even newborns too young for vaccines are shielded by the immune community around them.

Module Summary

- Innate: barriers, phagocytes, inflammation, complement, interferon — fast, no memory.
- Adaptive: B-cell antibodies (5 classes) + T-cell killing — specific, with memory.
- Vaccines: live, killed, toxoid, subunit; herd immunity protects all.
- Hypersensitivity types I-IV; serology uses antibody specificity.

Exam Pointers (Very Important)

- ★ Compare innate and adaptive immunity.
- ★ Describe antibody structure and the five classes.
- ★ Explain herd immunity and vaccine types.
- ★ What are the four types of hypersensitivity?

MODULE 7: MAJOR PATHOGENS: BACTERIA, VIRUSES, FUNGI, PARASITES

Learning Objectives

- Survey key bacterial pathogens by disease
- Explain viral replication and major viral diseases
- Describe fungal and parasitic infections
- Apply Koch's logic to emerging diseases

Bacterial Pathogens

Gram-positive: **Staphylococcus aureus** (skin infections, food poisoning, MRSA), **Streptococcus pyogenes** (pharyngitis, rheumatic fever), **Mycobacterium tuberculosis** (acid-fast, TB), **Clostridium** spp. (tetanus, botulism, gas gangrene — spore-formers). Gram-negative: **E. coli** (UTI, some strains cause dysentery), **Salmonella/Shigella** (enteric fever/dysentery), **Vibrio cholerae** (cholera toxin → watery diarrhea), **Neisseria** (gonorrhea, meningitis). **Virulence factors**: capsules, toxins (exo/endo), enzymes, adhesion.

Viruses

Obligate intracellular parasites: **lytic** cycle (replicate and burst out) vs. **lysogenic** (integrate as prophage, reactivate later). Cultivation needs **cell culture, embryonated eggs, or animals** — not agar plates. Major diseases: influenza, HIV, hepatitis (A-E), dengue, rabies, polio, measles, SARS-CoV-2. **Antibiotics do NOT work on viruses** — antivirals target specific replication steps; prevention is vaccination and vector control.

Fungi and Parasites

Fungi: **superficial** (dermatophytes — ringworm), **subcutaneous**, **systemic** (Aspergillus, Candida in immunocompromised), **opportunistic**. Treated with antifungals (target ergosterol in fungal membranes). Parasites: **protozoa** (Plasmodium — malaria via Anopheles mosquito; Entamoeba histolytica — amoebic dysentery; Giardia), **helminths** (roundworms, tapeworms — Ascaris, Taenia). Control = sanitation, vector control, clean water — public-health measures as much as drugs.

Key Terms & Definitions

Virulence factor	Microbial trait enabling disease (toxin, capsule...).
Lytic vs. lysogenic	Immediate replication vs. dormant integration.
Opportunistic pathogen	Causes disease mainly when immunity is weakened.
Vector	Organism transmitting a pathogen (e.g., mosquito).

Example: Cholera's Toxin Logic

Vibrio cholerae itself doesn't invade — its toxin locks intestinal cells' chloride channels open, pouring water into the gut: liters of watery diarrhea per day. Treatment is oral rehydration, not just antibiotics — replace the water and the patient survives while immunity clears the microbe. Understanding pathogenesis dictates therapy.

Module Summary

- Key bacteria: Staph, Strep, TB, Clostridium; E. coli, Salmonella, Vibrio, Neisseria.
- Viruses: lytic/lysogenic; need living cells to grow; antibiotics useless.
- Fungi: dermatophytes → systemic opportunists; parasites: protozoa + helminths.
- Virulence factors explain how; control mixes drugs with public health.

Exam Pointers (Very Important)

- ★ List four Gram-positive and four Gram-negative pathogens with diseases.
- ★ Contrast lytic and lysogenic cycles.
- ★ Why don't antibiotics work against viruses?
- ★ Describe the malaria life cycle and control measures.

MODULE 8: FOOD, INDUSTRIAL, AND ENVIRONMENTAL MICROBIOLOGY

Learning Objectives

- Explain food spoilage, preservation, and fermentation
- Describe industrial fermentations and products
- Explain microbes' role in nutrient cycles
- Describe water testing and wastewater treatment

Food Microbiology

Spoilage: microbial growth causing off-odors, textures, colors — controlled by temperature, pH, water activity. **Preservation:** heat (canning, pasteurization), cold (refrigeration/freezing), drying, high sugar/salt, acids, chemical preservatives, irradiation, MAP packaging. **Foodborne disease:** infection (Salmonella — microbe grows in host) vs. **intoxication** (Staph. aureus, Clostridium botulinum — preformed toxin ingested). **Fermented foods:** yogurt/cheese (Lactobacillus), bread/beer (yeast), pickles — controlled microbial activity as manufacture.

Industrial Microbiology

Microbes as factories: **antibiotics** (Penicillium, Streptomyces — secondary metabolites in stationary phase), **organic acids** (citric acid — Aspergillus niger), **amino acids**, **enzymes** (amylases, proteases), **biofuels** (ethanol), **recombinant proteins** (insulin in E. coli). **Strain improvement** by mutation/selection and genetic engineering; **fermenter** design controls aeration, pH, temperature for maximum yield.

Environmental Microbiology

Nutrient cycles are microbial: **nitrogen cycle** (fixation by Rhizobium, nitrification, denitrification), **carbon cycle** (decomposition), sulfur cycle. **Bioremediation:** microbes degrading oil spills, pesticides. **Water microbiology:** **coliform/ E. coli testing** indicates fecal contamination; **wastewater treatment** — primary (settling), secondary (**activated sludge** — aerobic microbes digest organics), tertiary (nutrient removal, disinfection). Safe water = applied microbiology saving millions of lives.

Key Terms & Definitions

Intoxication vs. infection	Preformed toxin ingested vs. pathogen growing in host.
Secondary metabolite	Non-growth compound (antibiotics) made in stationary phase.
Activated sludge	Aerobic microbial process digesting wastewater organics.
Coliform test	Indicator test for fecal contamination of water.

Example: Yogurt: Controlled Spoilage

Milk left warm spoils randomly; inoculated with *Lactobacillus*, the same milk becomes yogurt — lactic acid drops pH, preserving it and creating flavor. The difference between spoilage and manufacture is which microbe, controlled how. All food microbiology is this principle scaled up.

Module Summary

- Food: spoilage vs. preservation; infection vs. intoxication; fermentation as manufacture.
- Industry: antibiotics, acids, enzymes, biofuels, recombinant proteins from fermenters.
- Environment: N/C cycles are microbial; bioremediation cleans pollution.
- Water: coliform testing + activated-sludge treatment protect public health.

Exam Pointers (Very Important)

- ★ Distinguish food infection from food intoxication with examples.
- ★ List four preservation methods and their principles.
- ★ Describe the stages of wastewater treatment.
- ★ Explain the nitrogen cycle's microbial steps.

MODULE 9: LABORATORY TECHNIQUES AND BIOSAFETY

Learning Objectives

- Describe pure-culture techniques
- Explain staining and microscopy
- Outline culture media types and identification flow
- Apply biosafety levels and aseptic practice

Seeing Microbes

Light microscopy: bright-field (stained specimens), phase-contrast (live unstained), fluorescence (tagged antibodies — immunofluorescence), dark-field (spirochetes). **Electron microscopy** (TEM/SEM) resolves viruses and ultrastructure. **Stains:** simple, differential (**Gram**, acid-fast), structural (spore, capsule, flagella). Resolution limit of light microscopy ($\sim 0.2 \mu\text{m}$) explains why viruses need EM or molecular detection.

Growing and Identifying

Pure culture: streak plate, pour plate, spread plate from a single colony. Media: **defined vs. complex**; **selective** (favor one group — MacConkey for Gram-negatives), **differential** (distinguish by reaction — blood agar hemolysis), **enriched**. Identification flow: morphology → Gram stain → biochemical tests (catalase, oxidase, sugar fermentation, IMViC) → serology → **molecular** (PCR, 16S rRNA sequencing — the modern gold standard).

Biosafety

Aseptic technique: flame loops, sterile media handling, no food/drink, disinfect benches. **Biosafety levels:** BSL-1 (non-pathogens, teaching), BSL-2 (moderate pathogens — most clinical work; biosafety cabinet), BSL-3 (aerosol-transmitted serious disease — TB lab), BSL-4 (lethal, no treatment — Ebola; positive-pressure suits). **Waste:** autoclave before disposal; sharps in puncture-proof containers. Safety is not bureaucracy — it is the discipline that lets the science happen.

Key Terms & Definitions

Pure culture	Population of a single microbial species.
Selective vs. differential media	Favoring growth vs. distinguishing reactions.
16S rRNA sequencing	Molecular identification via ribosomal RNA gene.
BSL	Biosafety level 1-4 matching pathogen risk.

Example: From Colony to Name in 48 Hours

A throat swab streaked on blood agar: beta-hemolytic colonies → Gram-positive chains → catalase-negative → bacitracin-sensitive = *Streptococcus pyogenes*. Each test halves the possibilities; the flow chart turns a mixed swab into a named pathogen and a treatment decision.

Module Summary

- Microscopy: bright-field → fluorescence → EM; stains reveal structure.
- Pure culture via streak/pour/spread; media selective, differential, enriched.
- ID flow: morphology → Gram → biochemical → serology → molecular (16S/PCR).
- Biosafety: aseptic technique + BSL-1 to BSL-4 matched to risk.

Exam Pointers (Very Important)

- ★ Describe the streak-plate method for pure culture.
- ★ Compare selective and differential media with examples.
- ★ Outline a bacterial identification workflow.
- ★ What distinguishes BSL-2 from BSL-3 practice?

MODULE 10: ANTIMICROBIAL DRUGS AND RESISTANCE

Learning Objectives

- Classify antibiotics by mechanism
- Explain selective toxicity
- Describe resistance mechanisms
- Promote rational antibiotic use

How Antibiotics Work

Five targets: **cell-wall synthesis** (β -lactams — penicillins, cephalosporins; vancomycin), **protein synthesis** (70S ribosome — tetracyclines, aminoglycosides, macrolides, chloramphenicol), **nucleic acids** (fluoroquinolones — DNA gyrase; rifampin — RNA polymerase), **cell membrane** (polymyxins), **metabolic pathways** (sulfonamides — folic acid synthesis). **Selective toxicity**: drugs exploit microbe-specific targets (peptidoglycan, 70S ribosomes) — the basis of safe therapy. **Broad vs. narrow spectrum**: broad covers more but disrupts normal flora more.

Resistance Mechanisms

Bacteria fight back by: **enzymatic inactivation** (β -lactamases destroy penicillins), **target modification** (altered PBPs in MRSA; mutated gyrase), **efflux pumps** (pumping drug out), **decreased permeability** (porin loss in Gram-negatives), **alternative pathways** (bypassing blocked folate synthesis). **MRSA, VRE, ESBL, carbapenem-resistant** organisms are the clinical faces of these mechanisms. Resistance genes ride **plasmids and transposons** (see Module 5) — use selects for them.

Rational Use and Testing

Susceptibility testing: disk diffusion (Kirby-Bauer — zone sizes) and MIC determination guide therapy. Rational use: right drug, right dose, right duration; **complete the course**; don't use antibiotics for viral infections; reserve last-line drugs. **Antibiotic stewardship** programs + infection control + new-drug development are the three fronts of the resistance war. In Pakistan, over-the-counter antibiotic sales without prescription are a major driver — policy and behavior must change together.

Key Terms & Definitions

β-lactam	Antibiotic with a beta-lactam ring (penicillins, cephalosporins).
MIC	Minimum inhibitory concentration.
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i> .
Stewardship	Coordinated program promoting appropriate antibiotic use.

Example: The Zone That Decides

A Kirby-Bauer plate shows no zone around the penicillin disk but a wide zone around vancomycin: the *S. aureus* is penicillin-resistant, vancomycin-susceptible. Therapy follows the zones, not habits — susceptibility testing is the bridge between microbiology theory and the patient's bedside.

Module Summary

- Antibiotics hit wall, 70S ribosome, nucleic acids, membrane, folate pathway.
- Resistance: β -lactamases, target change, efflux, impermeability, bypass.
- Test with Kirby-Bauer/MIC; treat rationally; complete courses.
- Stewardship + infection control + new drugs = the response.

Exam Pointers (Very Important)

- ★ Classify antibiotics by mechanism with one example each.
- ★ Explain four resistance mechanisms.
- ★ What is selective toxicity? Give two examples.
- ★ Design an antibiotic stewardship poster — list five messages.