Microbiology, immunology and parasitology

MBBS Recap: high-yield notes for revision

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Revision notes for the MD/MS entrance: the facts examiners ask, set out for fast review after a wrong answer.

General bacteriology and sterilisation

Bacterial cell and Gram stain

- **Gram-positive**: thick, cross-linked peptidoglycan with teichoic and lipoteichoic acids; no outer membrane. Alcohol dehydrates the thick wall, the pores shrink and the **crystal violet-iodine complex is trapped**, so cells stay purple.
- **Gram-negative**: thin peptidoglycan plus an **outer membrane** with lipopolysaccharide (endotoxin: lipid A is the toxic part). Alcohol dissolves the outer membrane, the dye washes out and the safranin counterstain turns them pink.
- Acid-fastness depends on **mycolic acid** in the wall (Mycobacterium, Nocardia partially, Cryptosporidium and Isospora oocysts with a modified stain). It is not the reason for Gram positivity.
- **No cell wall**: Mycoplasma (unusual among bacteria in having sterols in its membrane, taken from the medium; stains poorly with Gram and is resistant to beta-lactams). Forms **"fried egg" colonies** on special agar (PPLO), the genus answer the exam expects; strictly the classic fried egg is M. hominis, while M. pneumoniae colonies are often granular (mulberry) without a clear halo at first. Ureaplasma and Mycoplasma pneumoniae are the examples to know. Chlamydia is an obligate intracellular organism and does not grow on agar.
- Capsule: polysaccharide (poly-D-glutamate in Bacillus anthracis). Encapsulated pathogens: Streptococcus pneumoniae, Haemophilus influenzae type b, Neisseria meningitidis, Klebsiella, Cryptococcus (a yeast). Spleen is vital for clearing them, so asplenic patients need vaccines against these.
- Spores: Bacillus and Clostridium (Geobacillus too). Dipicolinic acid and calcium make them heat resistant.

Special stains and what they show

Stain	Demonstrates
Albert	Metachromatic (volutin) granules of Corynebacterium diphtheriae: bluish-black granules in green cells
Ziehl-Neelsen	Acid-fast bacilli (M. tuberculosis decolourised with 20-25% sulphuric acid; M. leprae with weaker 5% acid; weak acid, 1%, for Nocardia and oocysts)
India ink / nigrosin	Capsule of Cryptococcus neoformans, seen as a clear halo; mucicarmine stains the capsule in tissue
Giemsa	Blood parasites, Borrelia, Chlamydia and Histoplasma, Leishmania
Auramine-rhodamine	Fluorescent screening for mycobacteria
Gomori methenamine silver	Fungi, Pneumocystis
Neisser	Diphtheria granules (older method)
Dark-field / silver (Fontana)	Treponema, Leptospira, which are too thin for Gram

- **Negative staining**: an acidic dye, **nigrosine** or India ink, is repelled by the negatively charged cell and colours only the background, so cells and capsules show clear; there is no heat fixation, so cells keep their true size and shape.
- **Bipolar ("safety-pin") staining** with Wayson, Giemsa or methylene blue: **Yersinia pestis** (the classic), also Pasteurella multocida, Burkholderia pseudomallei, Vibrio parahaemolyticus and the Donovan bodies of Klebsiella granulomatis.

Culture media

- **Selective**: TCBS (Vibrio; V. cholerae gives **yellow** sucrose-fermenting colonies, V. parahaemolyticus green); Löwenstein-Jensen (M. tuberculosis, egg-based, glycerol); modified **Thayer-Martin** (Neisseria gonorrhoeae and meningitidis, with vancomycin, colistin, nystatin); Bordet-Gengou or Regan-Lowe (Bordetella pertussis); Loeffler serum slope and tellurite (C. diphtheriae); MacConkey (lactose fermenters pink, non-fermenters colourless); **sorbitol-MacConkey** (E. coli O157:H7 does not ferment sorbitol); Salmonella-Shigella media: SS, XLD and DCA (thiosulphate with an iron salt shows H2S as black-centred colonies); **cetrimide agar** (a quaternary ammonium detergent) for Pseudomonas aeruginosa; **buffered charcoal yeast extract (BCYE)** with L-cysteine and iron for Legionella (which will not grow on blood agar; Nocardia also grows on it); Eaton or PPLO medium for Mycoplasma pneumoniae.
- Enrichment: selenite F, tetrathionate (Salmonella); alkaline peptone water (Vibrio).
- Enriched: chocolate agar (Haemophilus needs X and V factors; Neisseria); blood agar.
- **Mueller-Hinton agar** for susceptibility testing.
- Anaerobes: Robertson cooked meat broth, thioglycollate; Clostridium on Nagler's plate (lecithinase, C. perfringens).
- **Kirby-Bauer**: a standard inoculum spread as a **lawn** on Mueller-Hinton agar with antibiotic discs; the zone of inhibition is read against CLSI or EUCAST breakpoints. Streaking isolates colonies, pour plates count viable organisms, MIC by broth dilution or E-test (strip gradient).
- Campylobacter jejuni: curved rod with **darting motility**, microaerophilic, grown at **42°C** on Skirrow or CCDA medium.

Bacterial genetics

- **Transformation**: uptake of free, naked DNA (competent pneumococci, Haemophilus, Neisseria); Griffith's experiment.
- **Transduction**: DNA carried by a bacteriophage. Generalised (any gene) or specialised (genes next to the prophage). Toxins

carried by phage: diphtheria (beta phage), botulinum types C and D, the Shiga toxins of E. coli O157 (Stx1 and Stx2; the Shiga toxin of Shigella dysenteriae type 1 is chromosomal), cholera (CTX phage), erythrogenic (pyrogenic exotoxin A) toxin of Streptococcus pyogenes, Panton-Valentine leukocidin of S. aureus (mnemonic ABCDE: shigA-like, Botulinum, Cholera, Diphtheria, Erythrogenic). **Tetanus toxin is plasmid-encoded**, not phage-borne (also plasmid-borne: the anthrax toxins and capsule, E. coli LT and ST).

- **Conjugation**: cell-to-cell contact through a sex pilus; F plasmid; the **main route for spread of resistance (R) plasmids** in hospital Gram-negative flora, even between species, each plasmid often carrying several resistance genes at once.
- Lysogenic conversion: a change in the phenotype from an integrated prophage.
- Transposons move between plasmids and chromosome; integrons capture gene cassettes.

Sterilisation and disinfection

Method	Setting and use
Autoclave (moist heat under pressure)	**121°C, 15 psi, 15 minutes**; or 134°C, 30 psi, 3 minutes (prion-contaminated items use 134°C for 18 minutes). Kills spores. Surgical instruments, dressings, culture media.
Hot air oven (dry heat)	160°C for 1 hour holding time (2 hours in some texts), or 180°C for 30 minutes; glassware, oils, powders, sharps
Boiling	100°C does not kill spores; disinfection only
Pasteurisation	Holder 63°C for 30 minutes; flash 72°C for 15 seconds; not sterilisation
Tyndallisation	Steaming at 100°C for 20 minutes on three successive days; sugar and gelatin media
Inspissation	80-85°C for 30 minutes on 3 days; serum and egg media (LJ)
Gamma irradiation	Ionising cold sterilisation of sealed disposable plastics (syringes, gloves, catheters), sutures
Ethylene oxide, hydrogen peroxide plasma	Heat-sensitive items (endoscopes, plastics)
Glutaraldehyde 2%	High-level disinfection of endoscopes, 20 minutes; sterilisation with 10 hours
Membrane filtration	Heat-labile liquids; HEPA for air

- **Biological indicators**: **Geobacillus stearothermophilus** spores for the steam autoclave (and plasma); **Bacillus atrophaeus** (subtilis var. niger) for dry heat and ethylene oxide (classically, a non-toxigenic Clostridium tetani strain for the hot air oven, the older exam answer); Bacillus pumilus for radiation. Chemical indicators: Browne's tube, autoclave tape.
- Chemical: alcohol 70% (not sporicidal); chlorhexidine; povidone iodine; sodium hypochlorite 0.5-1% (5,000-10,000 ppm available chlorine) for blood spills (HIV, HBV); phenolics for TB sputum.
- **Spaulding classification** (CDC 2008 guideline): *critical* items entering sterile tissue or blood (surgical instruments, implants) need **sterilisation**; *semicritical* items touching mucous membranes or non-intact skin (flexible endoscopes, laryngoscope blades) need at least **high-level disinfection** (glutaraldehyde, ortho-phthalaldehyde, peracetic acid); *non-critical* items on intact skin (stethoscope, blood pressure cuff, bedpan) need low-level disinfection.
- Resistance to disinfection, most to least: **prions**, bacterial spores, coccidian oocysts (Cryptosporidium), mycobacteria, non-enveloped (non-lipid) small viruses, fungi, vegetative bacteria, enveloped (lipid) viruses least.
- Prions resist usual sterilisation; use 1 N NaOH or high-temperature autoclaving (134°C for 18 minutes).

Biosafety

- **BSL-1** non-pathogenic; **BSL-2** moderate-risk agents (HBV, HIV routine diagnostics, Salmonella); **BSL-3** airborne serious agents (M. tuberculosis culture, SARS-CoV-2 **culture**, Brucella culture, Coxiella); **BSL-4** lethal agents with no treatment (Ebola, Marburg, Lassa, Nipah, Crimean-Congo fever). WHO guidance: routine diagnostic work on clinical SARS-CoV-2 samples at BSL-2 with good practice; virus propagation at BSL-3.

Bacterial toxin groups worth remembering

- Endotoxin: LPS, heat stable; exotoxin: protein, mostly heat labile.
- AB toxins and ADP-ribosylation: **diphtheria** (EF-2), Pseudomonas exotoxin A (EF-2), cholera (Gs, raises cAMP), E. coli LT (Gs), pertussis (inhibits Gi).
- Superantigens: toxic shock syndrome toxin-1, staphylococcal enterotoxins, streptococcal pyrogenic exotoxins.
- Neurotoxins: tetanospasmin and botulinum toxin cleave SNARE proteins.

Infection basics: Koch, flora, nosocomial infection, pathogenesis

- **Koch's postulates:** the organism is found in every case and not in healthy people; it is grown in pure culture; the culture causes the same disease in a susceptible host; and it is re-isolated from that host. Exceptions: Mycobacterium leprae, Treponema pallidum, viruses (need cell culture), and carrier or asymptomatic infections. The **molecular Koch's postulates** (Falkow) test a virulence gene rather than the organism.
- **Normal flora:** skin (Staphylococcus epidermidis, Cutibacterium acnes, diphtheroids), mouth (viridans streptococci), upper airway (Neisseria, Haemophilus), colon (Bacteroides, anaerobes outnumber E. coli about 1000 to 1), vagina (lactobacilli keep pH 4–4.5), urethra. Flora give **colonisation resistance** and make vitamin K and some B vitamins; antibiotics disturb them and permit Clostridioides difficile and Candida overgrowth.
- **Nosocomial (healthcare-associated) infection** begins 48 hours or more after admission. **The commonest is urinary tract infection (mostly catheter-associated: the textbook answer)**, then surgical site infection, hospital and ventilator-associated pneumonia and central-line bloodstream infection; in recent prevalence surveys (CDC 2015, ECDC 2022–23) pneumonia and surgical site infection now lead in acute hospitals. Usual organisms: E. coli, Klebsiella, Pseudomonas aeruginosa, Staphylococcus aureus (MRSA), Acinetobacter, enterococci (VRE), C. difficile, Candida. Control: **hand hygiene is the single most effective measure**, standard precautions, bundles for lines and catheters, antibiotic stewardship and surveillance.
- **Transmission:** direct contact, droplet (larger than 5 µm, travels about 1 m: influenza, meningococcus, pertussis), airborne (5 µm or smaller: tuberculosis, measles, varicella),

faeco-oral, vector, vertical, parenteral. **Pathogenesis steps:** entry, adherence (pili, adhesins), evasion (capsule, protein A, antigenic variation, IgA protease), invasion, toxin production, damage, exit. **Endotoxin** (lipid A of LPS, heat-stable, TLR4, fever and shock) against **exotoxin** (protein, mostly heat-labile, toxoid-forming).

- **Bacteriocins** are protein antibiotics made by a bacterium and acting on closely related strains (colicins from E. coli; encoded on plasmids); pyocin typing of Pseudomonas and colicin typing were used in epidemiology. **Bacteriophages** (virulent: lytic; temperate: lysogenic, integrate as prophage) are used for phage typing, transduction, phage therapy and cloning vectors.

Microscopy and laboratory methods (practicals)

- **Bright-field** (stained smears; resolution about 0.2 µm, oil immersion 100×), **dark-field** (live spirochaetes such as Treponema), **phase contrast** (live unstained cells), **fluorescence** (auramine for TB, acridine orange, FITC antibody), **electron** (viruses). Hanging-drop preparation shows **motility** (true motility versus Brownian movement).
- **Special stains:** Gram (violet = Gram-positive); **Ziehl–Neelsen** (acid-fast, 20% sulphuric acid for M. tuberculosis, 5% for M. leprae); **Albert stain** (green–blue bacilli with blue-black metachromatic granules in Corynebacterium diphtheriae, Chinese-letter arrangement); **spore stain** (Schaeffer–Fulton: malachite green with heat, spores green and cells red; Dorner uses carbol fuchsin and nigrosin); **capsule** by negative staining with an India ink wet mount (Cryptococcus halo; Klebsiella) or Anthony stain.
- **Rapid biochemical tests: catalase** (bubbles with hydrogen peroxide: positive in staphylococci, negative in streptococci), **oxidase** (tetramethyl-p-phenylenediamine turns purple: Pseudomonas, Neisseria, Vibrio positive; Enterobacterales negative), **coagulase** (slide bound test, tube free coagulase: S. aureus positive, coagulase-negative staphylococci negative). Others: indole, citrate, urease (Proteus, Klebsiella, H. pylori), triple sugar iron agar (acid slant and butt, gas, H2S).
- **Anaerobic culture:** the anaerobic jar (catalyst of palladium plus hydrogen and carbon dioxide from a gas-generating pack; the redox indicator methylene blue becomes colourless), anaerobic chambers, reducing media (cooked meat, thioglycollate), and transport of the specimen in anaerobic transport medium without delay. Strict anaerobes do not grow in air because they lack superoxide dismutase and catalase.
- **Sterilisation checks:** autoclave 121 °C for 15 minutes at 15 lb pressure with Bacillus stearothermophilus (Geobacillus) spore strips; hot-air oven 160 °C for 2 hours with spores of a non-toxigenic strain of Clostridium tetani (the classic answer) or Bacillus atrophaeus (formerly B. subtilis var. niger, the spore strip now used); Chick–Martin and Rideal–Walker coefficients test disinfectants. **Disinfection** inactivates most organisms; **decontamination** makes articles safe to handle; sterilisation kills all organisms including spores.
- **Cerebrospinal fluid (diagnosis of meningitis):**

	Normal	Bacterial (pyogenic)	Viral (aseptic)	Tuberculous	Fungal (cryptococcal)
Appearance	Clear	**Turbid**	Clear	Clear or opalescent, cobweb clot	Clear
Cells (per µL)	0–5 lymphocytes	**100–10,000+ neutrophils**	10–500 **lymphocytes**	10–500 lymphocytes (neutrophils early)	10–200 lymphocytes
Protein	15–45 mg/dL	**High**	Normal or mildly high	**High**	High
Glucose (CSF : blood ratio)	60% (about 0.6)	**Low (below 0.4)**	Normal	**Low**	Low or normal

Gram stain finds the organism in 60–90% before antibiotics; latex agglutination and PCR panels help in partly treated cases; **India ink** for Cryptococcus (with cryptococcal antigen); ZN stain, adenosine deaminase and Xpert MTB/RIF Ultra for TB; antibiotics are never delayed for the lumbar puncture: blood cultures, then antibiotics within an hour, and if CT must come first (focal signs, reduced consciousness, papilloedema, immunosuppression) antibiotics are given before it (ESCMID 2016; NICE 2024). Herpes simplex encephalitis: lymphocytes with red cells, HSV PCR positive. Collect CSF in three tubes (biochemistry, microbiology, cells).

- **Laboratory diagnosis of viral disease:** direct detection (PCR, antigen tests, electron microscopy, immunofluorescence), **isolation** in cell culture (cytopathic effect: syncytia with RSV and measles, rounding and clumping with adenovirus), embryonated hen's egg (allantoic cavity for influenza; chorioallantoic membrane for pox and herpes pocks; amniotic cavity for influenza; yolk sac for chlamydia), and animal inoculation (suckling mice for coxsackie and arboviruses), and **serology** (IgM or a four-fold rise in paired IgG). Inclusion bodies are a clue (see Virology).
- **Molecular diagnostics:** PCR and real-time PCR, reverse transcription PCR for RNA viruses, NAAT cartridges (Xpert), sequencing and probes; they detect non-culturable organisms and resistance genes.

Immunology and vaccines

Antibodies

Class	Key facts
IgG	Commonest in serum; the only class that **crosses the placenta** (neonatal Fc receptor); four subclasses; opsonises, activates complement (IgG1, IgG3 best); secondary response
IgM	Pentamer, first in primary response; best complement activator; **cannot cross the placenta**, so raised IgM in cord blood means fetal infection (TORCH)
IgA	Monomer in serum; secretory dimer with J chain and secretory piece; mucosal defence, colostrum and breast milk; commonest immunodeficiency is selective IgA deficiency (anaphylaxis to blood products with IgA)
IgE	Bound to mast cell and basophil Fc-epsilon receptors; type I hypersensitivity, helminths; lowest serum level
IgD	B-cell surface receptor with IgM

- Class switching needs helper T cells (CD40 ligand and cytokines): IL-4 gives IgE, IFN-gamma gives IgG, TGF-beta gives IgA.

- **Protein antigens** are T-dependent: presented on MHC II to helper T cells, so there is affinity maturation, class switching and memory. **Pure polysaccharides** are T-independent: cross-link B-cell receptors, give mainly IgM and poor memory, and fail in infants under 2. **Conjugating** the polysaccharide to a protein carrier (Hib, pneumococcal conjugate, meningococcal conjugate, typhoid conjugate) makes it T-dependent.
- Antibody classes in infection: IgM marks recent infection; IgG persists. In hepatitis B, IgM anti-HBc marks acute infection.

Cells and MHC

- MHC I (HLA-A, B, C): all nucleated cells, presents endogenous peptide to CD8; MHC II (DP, DQ, DR): antigen-presenting cells, presents exogenous peptide to CD4. Cytotoxic CD8 kill by perforin and granzyme; NK cells kill cells lacking MHC I.
- **Lymphoid zones**: the lymph node **paracortex** is the T-cell zone (depleted in DiGeorge syndrome), follicles and germinal centres are B-cell areas; in the spleen the periarteriolar lymphoid sheath holds T cells. **After splenectomy** the film shows **Howell-Jolly bodies** (nuclear remnants the spleen normally removes), Pappenheimer bodies, target cells and thrombocytosis, and the risk of overwhelming sepsis by encapsulated organisms (pneumococcus, Hib, meningococcus) rises, so vaccinate (ideally 2 weeks before elective splenectomy).
- Th1 (IFN-gamma, IL-2; intracellular organisms, type IV); Th2 (IL-4, 5, 13; helminths, allergy); Th17 (IL-17; neutrophils, fungi); Treg (FoxP3, IL-10).
- **Molecular mimicry**: cross-reactivity between a microbial antigen and a self antigen. Rheumatic fever: antibodies against group A streptococcal **M protein** react with cardiac myosin and valve antigens; Guillain-Barré after Campylobacter (GM1 gangliosides).

Complement

- Classical (C1q on IgG or IgM complexes), alternative (spontaneous C3 hydrolysis, properdin), lectin (mannose-binding lectin). C3 is central; C3b opsonises; C5a is the main chemotaxin and anaphylatoxin (with C3a).
- **Membrane attack complex C5b-C9** lyses Gram-negative cells: its deficiency gives **recurrent Neisseria** infections (meningococcal, gonococcal). C3 deficiency: severe recurrent pyogenic infections. C1 esterase inhibitor deficiency: hereditary angioedema (low C4). DAF (CD55) and CD59 deficiency: paroxysmal nocturnal haemoglobinuria. Early classical component (C1, C2, C4) deficiency: SLE-like disease.

Hypersensitivity (Gell and Coombs)

Type	Mechanism	Examples
I	IgE on mast cells; immediate	Anaphylaxis, asthma, hay fever, food allergy
II	Antibody against cell-surface or matrix antigen	Autoimmune haemolytic anaemia, Goodpasture, myasthenia (functional), Graves, rheumatic carditis, transfusion reactions
III	**Immune complex deposition**, complement activation	**Serum sickness** (1-2 weeks after foreign protein such as antivenom, fever, rash, arthralgia, low complement), Arthus reaction (local), post-streptococcal GN, SLE, polyarteritis
IV	**T-cell mediated, delayed** (48-72 hours)	**Tuberculin test**, contact dermatitis, granuloma, graft rejection, multiple sclerosis, type 1 diabetes

- Tuberculin (Mantoux): 5 TU PPD-S (or 2 TU PPD RT23, the equivalent dose used in South Asia) intradermally, read at 48-72 hours by transverse induration. Cut-off **5 mm or more** in HIV-positive, recent contacts, immunosuppression; 10 mm in other risk groups; 15 mm in low risk. A false negative is common in anergy and miliary TB. IGRA (QuantiFERON, T-SPOT) is not affected by BCG.
- Anaphylaxis: adrenaline intramuscular 0.5 mg (adult) is first line.

Immunodeficiency

- **X-linked (Bruton) agammaglobulinaemia**: **BTK mutation**, block at pre-B to B maturation, absent CD19+ B cells, all immunoglobulins very low, boys from 6 months (after maternal IgG wanes) with recurrent infections by encapsulated pyogenic bacteria and enteroviruses; small tonsils and nodes; treated with IVIG.
- Hyper-IgM syndrome: **CD40 ligand** defect, normal B cells, raised IgM, low IgG, IgA and IgE.
- DiGeorge: 22q11 deletion, thymic aplasia, hypocalcaemia, cardiac defects, low T cells.
- SCID: ADA deficiency (accumulation of deoxyadenosine toxic to lymphocytes), IL-2R gamma chain (X-linked), RAG defects. **Live vaccines (BCG, oral polio, MMR, varicella, rotavirus) are contraindicated** because the vaccine strain multiplies unchecked.
- Wiskott-Aldrich: WAS gene, eczema, thrombocytopenia (small platelets), infections.
- Chronic granulomatous disease: NADPH oxidase defect, catalase-positive organisms (S. aureus, Aspergillus, Nocardia, Burkholderia), abnormal nitroblue tetrazolium or DHR test.
- Chediak-Higashi: LYST gene, giant granules, albinism. Leukocyte adhesion deficiency: CD18, delayed cord separation, no pus. Common variable immunodeficiency: adults with low IgG and IgA.

Transplant, transfusion, autoimmunity and tests

- **Hyperacute rejection** (minutes to hours after the vessels are unclamped; graft blue, mottled and flaccid): preformed recipient antibody to ABO or HLA antigens, complement and thrombosis (type II), irreversible; prevented by ABO matching and a negative crossmatch. Acute rejection: mainly T cells, days to weeks (an antibody-mediated form too). Chronic: graft arteriopathy (intimal thickening) and fibrosis over months to years.
- **Graft-versus-host disease** after allogeneic marrow or stem-cell transplant: **donor T cells** attack host tissue, the **skin first** (maculopapular rash, often palms and soles), then gut (diarrhoea) and liver (rising bilirubin); the same alloreactivity gives a useful graft-versus-leukaemia effect. Classically acute within 100 days; current criteria (NIH consensus 2014) separate acute and chronic by their features, not the day. Also after non-irradiated transfusion in the immunocompromised (prevented by irradiating the blood).
- **Transfusion reactions**: **acute haemolytic** (ABO mismatch, usually a clerical error; type II, within minutes); **febrile non-haemolytic** (the commonest; during or within 4 hours; recipient anti-HLA antibody against donor leucocytes plus

cytokines accumulating in stored blood; pre-storage leucodepletion lowers it); **TRALI** (within 6 hours; donor anti-HLA or anti-neutrophil antibody; non-cardiogenic pulmonary oedema with normal or low venous pressure, often hypotension and fever); **TACO** (volume overload, within 12 hours, ISBT 2018 criteria; the elderly and those with heart or kidney failure; hypertension, raised JVP and BNP, responds to diuretics); anaphylaxis in IgA deficiency with anti-IgA antibodies.

- **Autoantibodies**: anti-topoisomerase I (**Scl-70**) diffuse cutaneous systemic sclerosis (with lung fibrosis); **anticentromere** limited disease (CREST); anti-RNA polymerase III diffuse disease with renal crisis; **anti-U1 RNP** mixed connective tissue disease; anti-Jo-1 antisynthetase myositis; anti-Smith and anti-dsDNA specific for SLE; anti-histone drug-induced lupus. **Anti-desmoglein 3** (with or without desmoglein 1) pemphigus vulgaris: flaccid blisters, Nikolsky positive, suprabasal acantholysis; **anti-BP180 (hemidesmosome)** bullous pemphigoid: tense subepidermal blisters; anti-type VII collagen epidermolysis bullosa acquisita.
- HLA associations: B27 (ankylosing spondylitis), DR3 and DR4 (type 1 diabetes), DR4 (rheumatoid arthritis), B5 (Behcet), DQ2 and DQ8 (coeliac).
- ELISA, Western blot (once the HIV confirmatory test, now replaced by a second differentiating immunoassay and nucleic acid tests in current algorithms), flow cytometry (CD markers), immunofluorescence, PCR.

Vaccines

Type	Examples
Live attenuated	BCG, oral polio (Sabin), measles, MMR, rubella, varicella, yellow fever (17D), oral typhoid Ty21a, rotavirus, intranasal influenza
Killed or inactivated	Salk (injectable) polio, rabies, hepatitis A, whole-cell pertussis, cholera (oral killed), Japanese encephalitis (inactivated Vero-cell vaccine; a live attenuated JE vaccine, SA 14-14-2, also exists)
Toxoid	**Tetanus**, diphtheria (formaldehyde-treated toxins, protect against toxin, not the organism)
Subunit and recombinant	Hepatitis B (recombinant HBsAg), HPV (L1 virus-like particles), acellular pertussis
Polysaccharide	Pneumococcal 23-valent, Vi typhoid, meningococcal
Conjugate	Hib, PCV, meningococcal ACWY, typhoid conjugate
mRNA and vector	COVID-19 (mRNA, adenoviral vector)

- The attenuated oral polio vaccine can cause vaccine-associated paralysis and circulating vaccine-derived poliovirus, so the injectable inactivated vaccine is used alongside, with the oral type 2 component withdrawn (2016 switch from trivalent to bivalent).
- Cold chain: **OPV is the most heat-sensitive vaccine** and may be frozen (stored frozen at central level); BCG and measles-rubella are freeze-dried and light sensitive (reconstituted vials used within 6 hours); freeze-sensitive vaccines (hepatitis B, DPT and pentavalent, tetanus-diphtheria, Hib, pneumococcal, IPV) must never be frozen, and the **shake test** shows whether an aluminium-adsorbed vaccine has been frozen. The vaccine vial monitor shows cumulative heat exposure.
- Nepal runs a national immunisation programme with its own schedule; the exact schedule changes with new introductions, so use the current Nepal schedule rather than a textbook's (generally from Indian or Western tables). Two recent points: Japanese encephalitis vaccine is given at 12 months, and **typhoid conjugate vaccine** entered the routine schedule at 15 months after a nationwide campaign for children under 15 in 2022 (Gavi and UNICEF, 2022).

Systemic bacteriology

Gram-positive cocci

- **Catalase**: staphylococci positive, streptococci and enterococci **negative** (the first test to split a chain-forming Gram-positive coccus). Coagulase positive: **Staphylococcus aureus**. Novobiocin-resistant coagulase-negative: S. saprophyticus (honeymoon cystitis in young women); S. epidermidis colonises catheters and prosthetic valves through biofilm.
- S. aureus: protein A binds IgG Fc; toxins cause food poisoning (preformed enterotoxin, 1-6 hours), toxic shock (TSST-1), scalded skin (exfoliatin). MRSA carries **mecA** (PBP2a): treat with vancomycin, linezolid, daptomycin (not for pneumonia).
- **Streptococcus pneumoniae**: lancet-shaped alpha-haemolytic diplococci, **optochin sensitive, bile soluble**, quellung positive. Viridans streptococci: optochin resistant, bile insoluble (S. mutans causes caries; S. sanguinis endocarditis after dental work). Commonest cause of community-acquired pneumonia, otitis media, and meningitis in adults.
- Group A (S. pyogenes): beta-haemolytic, bacitracin sensitive, PYR positive. Pharyngitis, impetigo, scarlet fever, necrotising fasciitis. Non-suppurative sequelae: **rheumatic fever** (follows pharyngitis, by molecular mimicry with M protein; no sequel after skin infection) and post-streptococcal glomerulonephritis (after throat or skin; immune complexes). ASO titre rises after throat infection; anti-DNase B after skin.
- Group B (S. agalactiae): hippurate positive, CAMP positive; neonatal sepsis and meningitis; maternal screening by vaginal-rectal swab at **35-37 weeks** (CDC 2010, the textbook answer; ACOG 2020 now advises 36+0 to 37+6 weeks), intrapartum penicillin for carriers.
- Enterococci: bile-esculin positive, grow in 6.5% NaCl, intrinsically cephalosporin resistant; VRE.
- Streptococcus bovis (gallolyticus): endocarditis linked to colon cancer.

Gram-positive bacilli

- **Corynebacterium diphtheriae**: club-shaped, Chinese letter arrangement, **Albert stain** granules; Loeffler serum slope, tellurite agar (black colonies). **Diphtheria toxin** (phage-coded tox gene) **ADP-ribosylates elongation factor-2**, halting protein synthesis. Pseudomembrane on tonsils, bull neck, myocarditis. Treat with antitoxin plus penicillin or erythromycin; Schick test (historical).
- **Clostridium tetani**: terminal spore (drumstick). **Tetanospasmin** travels retrogradely, cleaves synaptobrevin and blocks release of **glycine and GABA** from Renshaw inhibitory interneurons, so spastic paralysis, trismus, risus sardonicus, opisthotonos. **Botulinum toxin** blocks acetylcholine release at the neuromuscular junction (flaccid paralysis, descending). Both are zinc proteases that cleave SNARE proteins.
- **Botulism**: preformed toxin in home-canned or preserved food (foodborne), spores in honey (infant botulism, floppy baby), or wound botulism; symmetrical **descending flaccid paralysis**

starting with cranial nerves (diplopia, ptosis, dysphagia), dry mouth and often dilated pupils, no fever and a clear sensorium; treat with antitoxin early and ventilatory support. Management of tetanus: human tetanus immunoglobulin, metronidazole, muscle relaxants, airway care.

- C. perfringens: gas gangrene (alpha toxin lecithinase, Nagler reaction), food poisoning. C. difficile: antibiotic-associated colitis, toxins A and B, diagnosed by GDH plus toxin assay or PCR; oral vancomycin or fidaxomicin first line; faecal transplant for recurrences.
- Bacillus anthracis: **capsule of poly-D-glutamate (polypeptide, unusual among bacteria; plasmid pXO2)**, "medusa head" colonies, McFadyean reaction; ciprofloxacin or doxycycline. Bacillus cereus: fried-rice emetic syndrome.
- Listeria monocytogenes: tumbling motility at 25°C, cold enrichment, neonates, pregnancy, elderly; ampicillin (cephalosporins do not cover it).
- Nocardia: weakly acid-fast branching filaments, cotrimoxazole. Actinomyces: sulphur granules, penicillin.

Gram-negative cocci and coccobacilli

- **Neisseria gonorrhoeae**: Gram-negative diplococci within neutrophils; ferments glucose only; urethritis, PID, septic arthritis; culture on **modified Thayer-Martin** (chocolate agar with VCN) in CO2; NAAT is now standard. Treatment: a single intramuscular dose of **ceftriaxone**, because resistance to quinolones and penicillin is widespread: **500 mg** (CDC 2021; 1 g if 150 kg or more), **1 g** (WHO 2024, BASHH 2025); the older 250 mg plus azithromycin 1 g dual therapy is superseded (doxycycline is added only if chlamydia is not excluded). Cefixime 800 mg orally with a test of cure if ceftriaxone cannot be given (WHO 2024). N. meningitidis ferments glucose and maltose; vaccine and chemoprophylaxis for contacts (rifampicin, ciprofloxacin or ceftriaxone).
- Haemophilus influenzae: needs X and V factors (satellitism around S. aureus); Hib vaccine has reduced meningitis and epiglottitis.
- **Bordetella pertussis**: Bordet-Gengou or Regan-Lowe medium; pertussis toxin ADP-ribosylates Gi; paroxysmal cough with whoop, lymphocytosis; macrolides.
- Brucella: small Gram-negative coccobacillus, Castaneda medium, undulant fever; doxycycline plus rifampicin or streptomycin; BSL-3 for culture. Legionella: charcoal yeast extract, urinary antigen; azithromycin or levofloxacin.
- **Animal bites**: **Pasteurella multocida** (small Gram-negative coccobacillus, oxidase positive, usually no growth on MacConkey) causes rapid cellulitis within 24 hours of a cat or dog bite; amoxicillin-clavulanate is the empirical bite treatment, penicillin the drug once it is isolated. *Capnocytophaga canimorsus* follows dog bites, with fulminant sepsis in asplenic people; *Bartonella henselae* causes cat-scratch lymphadenopathy over weeks; *Eikenella corrodens* is the human-bite (clenched-fist) organism.

Enterobacterales and enteric pathogens

- Lactose fermenters (pink on MacConkey): E. coli, Klebsiella, Enterobacter. Non-lactose fermenters: Salmonella, Shigella, Proteus, Pseudomonas, Yersinia.
- E. coli pathotypes: ETEC (travellers' diarrhoea, LT and ST toxins), EPEC (infantile diarrhoea), EIEC (dysentery), **EHEC O157:H7 (Shiga toxin, bloody diarrhoea, haemolytic uraemic syndrome; no antibiotics; non-sorbitol fermenter)**, EAEC (persistent diarrhoea). Uropathogenic E. coli: commonest cause of UTI. Klebsiella: currant-jelly sputum, pneumonia in alcoholics, liver abscess.
- **Enteric fever** (Salmonella Typhi and Paratyphi): step-ladder fever, relative bradycardia, rose spots in the second week, splenomegaly, leucopenia; **blood culture** is most sensitive in the first week (bone marrow culture is the most sensitive overall); Widal (O and H antibodies) from the second week, limited by cross-reactions and endemic background; stool and urine culture in later weeks; carriers harbour bacteria in the gallbladder. Treatment: azithromycin (uncomplicated) or ceftriaxone, because **fluoroquinolone non-susceptibility** is the rule in South Asia (about 87% of S. Typhi in Nepal, 95% in Pakistan and 98% in Bangladesh, Surveillance for Enteric Fever in Asia Project 2016-2019); classic multidrug resistance (ampicillin, chloramphenicol, cotrimoxazole) has become uncommon in Nepal. **Extensively drug-resistant (XDR) typhoid**, resistant to ceftriaxone and fluoroquinolones as well, emerged in Sindh, Pakistan in 2016 and is treated with azithromycin or a carbapenem; ceftriaxone resistance elsewhere is sporadic, and azithromycin-resistant isolates (acrB mutation) were reported from Kathmandu in 2019. Typhoid conjugate vaccine is recommended by WHO (position paper 2018) and has been in Nepal's routine schedule since 2022.
- Shigella: non-motile, no H2S, dysentery from tiny inocula; treated with ciprofloxacin, azithromycin or ceftriaxone by local resistance.
- **Vibrio cholerae**: comma-shaped, darting motility, TCBS yellow, O1 (classical and El Tor) and O139; cholera toxin ADP-ribosylates Gs (cAMP rises); rice-water stool; oral rehydration plus doxycycline or azithromycin; oral killed vaccine used in outbreaks.
- **Campylobacter jejuni**: curved rod, 42°C, the commonest bacterial cause of diarrhoea in high-income countries (in low-income settings ETEC, Shigella and Campylobacter all rank high), linked to Guillain-Barré syndrome and reactive arthritis. Yersinia enterocolitica: mesenteric adenitis mimicking appendicitis, cold enrichment.
- **Helicobacter pylori**: curved microaerophile, produces abundant **urease** (urea to ammonia and CO2), detected by rapid urease test, urea breath test, stool antigen (stop PPI two weeks and antibiotics or bismuth four weeks before); urea breath test is the non-invasive test of choice and of cure; associated with duodenal ulcer, gastric adenocarcinoma, MALT lymphoma (an IARC group 1 carcinogen). **Exam answer**: triple therapy, a PPI with clarithromycin and amoxicillin (metronidazole if penicillin allergic). **Current practice**: where clarithromycin resistance exceeds 15% or is unknown, **bismuth quadruple therapy** (PPI, bismuth, tetracycline, metronidazole) for **14 days** (Maastricht VI/Florence consensus 2022; ACG guideline, Chey et al., Am J Gastroenterol 2024, where it is the preferred first line for every treatment-naive patient, with rifabutin triple or vonoprazan-amoxicillin dual therapy as alternatives); clarithromycin triple therapy only when susceptibility is proven.
- Pseudomonas aeruginosa: oxidase positive, blue-green pigment (pyocyanin), grape smell; burns, ventilator pneumonia, cystic fibrosis; anti-pseudomonal beta-lactams.
- Proteus: swarming, urease positive, struvite stones. Acinetobacter: ICU infections, multidrug resistance.

Mycobacteria, spirochaetes, rickettsiae, chlamydiae

- **M. tuberculosis**: acid-fast, slow, Löwenstein-Jensen (grows in 4-8 weeks), liquid MGIT (1-3 weeks); **Xpert MTB/RIF** (and Ultra) gives rifampicin resistance in two hours by detecting mutations in the 81-base-pair core region of **rpoB** (the gene for the RNA polymerase β subunit that rifampicin binds); WHO 2022 guidelines prefer rapid molecular tests as the initial test. Cord factor, sulphatides. First-line: HRZE for 2 months then HR for 4 (exam answer, still standard); WHO 2022 also allows a 4-month regimen (2HPZM/2HPM, rifapentine-moxifloxacin) for people aged 12 and over, and 4 months (2HRZ(E)/2HR) for children with non-severe TB. The 6-month **BPaLM** regimen (bedaquiline, pretomanid, linezolid, moxifloxacin) is the WHO 2022 option for MDR/rifampicin-resistant TB.
- **Tuberculin test reading** (CDC): induration **5 mm or more is positive in HIV infection**, recent contacts of TB, fibrotic changes on chest X-ray and other immunosuppression; 10 mm or more in other risk groups (recent immigrants from endemic countries, injecting drug users, health workers, children under 4); 15 mm or more in people with no risk factor. BCG history does not change the cut-off in an HIV-positive patient.
- **M. leprae**: cannot be grown on artificial media; grows in the **footpad of the mouse** and the **nine-banded armadillo** (cool tissue). Bacillary index; tuberculoid (Th1, few bacilli) and lepromatous (Th2, many bacilli); WHO multidrug therapy with rifampicin, clofazimine and dapsone: since WHO 2018 the same three drugs for both forms, 6 months for paucibacillary and 12 months for multibacillary (the older exam answer gave paucibacillary disease rifampicin and dapsone only); single-dose rifampicin as post-exposure prophylaxis for contacts (WHO 2018).
- Atypical mycobacteria: M. avium complex (HIV with CD4 below 50), M. kansasii, M. marinum (fish tank), M. ulcerans (Buruli ulcer), rapid growers.
- **Treponema pallidum**: cannot be cultured; seen by dark-field. **VDRL and RPR** are non-treponemal flocculation tests that use **cardiolipin-cholesterol-lecithin** antigen; they fall with treatment and measure activity, but are falsely positive in SLE, antiphospholipid syndrome, pregnancy, malaria, leprosy and viral infections. **FTA-ABS, TPHA and TPPA** are treponemal, usually remain positive for life and confirm. In primary syphilis **FTA-ABS is the first to become positive** (the exam answer); in current laboratories treponemal enzyme or chemiluminescence immunoassays are as early and are used to screen (the reverse algorithm). Primary syphilis: painless chancre; secondary: rash and condylomata lata; tertiary: gumma, aortitis, tabes dorsalis (lightning pains, sensory ataxia, absent ankle jerks, **Argyll Robertson pupils**: small, irregular, constrict on accommodation but not to light). Congenital: Hutchinson teeth, mulberry molars, saddle nose, rhagades, sabre shins. Treatment: benzathine penicillin G. Prozone effect gives a false negative VDRL.
- Borrelia: Lyme (Ixodes tick), relapsing fever (louse, antigenic variation). **Leptospira**: Weil disease, microscopic agglutination test, penicillin or doxycycline.
- **Weil-Felix**: Proteus OX19 (epidemic and murine typhus, spotted fevers), OX2 (spotted fever group), **OXK (scrub typhus, Orientia tsutsugamushi)**. Scrub typhus is one of the main causes of acute undifferentiated fever in Nepal, recognised there mainly since the 2015 outbreaks after the earthquake (about a fifth of such fevers in pooled Nepali serosurveys, beside enteric fever, the other leading cause), peaking in the monsoon and after it; treat with doxycycline (azithromycin in pregnancy and young children); an eschar is the clue. Weil-Felix is insensitive; IgM ELISA is better.
- Chlamydia trachomatis: obligate intracellular, elementary and reticulate bodies; serovars A-C trachoma, D-K genital infection, L1-L3 lymphogranuloma venereum; NAAT diagnosis; doxycycline. **Fitz-Hugh-Curtis syndrome**: perihepatitis with violin-string adhesions between liver capsule and diaphragm, from pelvic infection by Chlamydia trachomatis or Neisseria gonorrhoeae; right upper quadrant pain, often pleuritic, with liver tests usually normal. Chlamydophila pneumoniae and psittaci cause atypical pneumonia.
- Coxiella: Q fever, no rash, culture-negative endocarditis.

Antibiotic resistance

- Beta-lactamases; ESBL (cephalosporin resistant, carbapenem treated); carbapenemases (NDM-1, KPC, OXA-48); MRSA (mecA), VRE (vanA); efflux and target modification.
- Respiratory atypicals (Mycoplasma, Chlamydia, Legionella) respond to macrolides, doxycycline, fluoroquinolones, not beta-lactams. **Mycoplasma** has **no cell wall** (hence beta-lactam resistance), sterols in its membrane, and grows on PPLO agar as tiny **"fried egg"** colonies (dense centre, lighter edge); M. pneumoniae causes walking pneumonia with cold agglutinins (IgM anti-I), and macrolide resistance is common in East Asia.

Bacteria named in the MBBS curriculum: facts to revise

- **Bacterial vaginosis (Gardnerella vaginalis with anaerobes):** thin grey homogeneous discharge with a **fishy odour; vaginal pH above 4.5; positive whiff test with KOH; clue cells** (epithelial cells coated with coccobacilli); these are the **Amsel criteria (3 of 4 diagnose)**, while Nugent scoring on a Gram stain is the laboratory standard; lactobacilli fall. Gardnerella is a Gram-variable pleomorphic coccobacillus. It is not a classical sexually transmitted infection; treat with metronidazole (oral or gel) or clindamycin; treating the male partner does not help (the exam answer); current evidence: treating regular male partners with oral metronidazole and topical clindamycin halved recurrence in a 2025 randomised trial (Vodstrcil et al., NEJM 2025), and guidelines are being revised to include it.
- **Haemophilus ducreyi:** chancroid, painful soft ulcer with ragged edges and tender suppurative inguinal bubo, Gram-negative coccobacilli in "school of fish" chains, grown on chocolate agar with vancomycin; treat with azithromycin or ceftriaxone.
- **Chlamydia trachomatis: obligate intracellular** (cannot make its own ATP: "energy parasite"), so it needs cell culture (McCoy cells), yolk sac or NAAT. **Life cycle:** infectious, metabolically inert **elementary body** enters the host cell by endocytosis, differentiates into the metabolically active **reticulate body**, which divides by binary fission inside an inclusion (Halberstaedter–Prowazek body, glycogen-positive), then reorganises into new elementary bodies and releases them. Not detectable by Gram stain (Giemsa or immunofluorescence instead); no peptidoglycan layer of the classical kind; treated with doxycycline 100 mg twice daily for 7 days (first choice, CDC 2021) or a single 1 g dose of azithromycin (in pregnancy, or when adherence is doubtful).

- **Pseudomonas aeruginosa:** strictly aerobic Gram-negative rod, **oxidase positive, non-lactose fermenter**, pigments **pyocyanin (blue-green) and pyoverdin (fluorescent green)**, **grape-like (fruity) odour**; burns and wound infection, ventilator pneumonia, ear (malignant otitis externa in diabetics), folliculitis from hot tubs, cystic fibrosis (mucoid alginate); intrinsically resistant, so treat by antipseudomonal agents (piperacillin–tazobactam, ceftazidime, cefepime, meropenem, ciprofloxacin, aminoglycosides, colistin for resistant strains). Exotoxin A inactivates eEF-2 like diphtheria toxin.
- **Neisseria meningitidis:** Gram-negative diplococci (kidney-shaped), oxidase positive, **ferments glucose and maltose** (gonococcus only glucose); capsule groups A, B, C, W, Y, X; droplet spread; petechial or purpuric rash and Waterhouse–Friderichsen syndrome; diagnosis by Gram stain of CSF, culture on chocolate agar and Thayer–Martin in CO_2, PCR; ceftriaxone, rifampicin or ciprofloxacin for contacts; vaccines (conjugate ACWY, protein B). Complement terminal-component deficiency predisposes to recurrent infection.
- **Corynebacterium diphtheriae:** Albert stain and Loeffler serum slope (colonies in 6–8 hours), tellurite blood agar (black colonies), **Elek gel precipitation test for toxigenicity** (toxin ADP-ribosylates elongation factor 2); grey pseudomembrane; antitoxin plus penicillin or erythromycin.
- **Bordetella pertussis:** tiny Gram-negative coccobacillus; **Bordet–Gengou (potato-blood-glycerol) agar** with colonies like mercury drops, or **Regan–Lowe (charcoal) medium** with cephalexin; the culture is best in the catarrhal stage from a nasopharyngeal swab (not throat swab); PCR is now commonly used; by the paroxysmal stage culture is often negative, as the cough is toxin-mediated and outlasts the bacteria.
- **Clostridium perfringens (gas gangrene):** large boxcar Gram-positive bacilli without spores seen; **alpha toxin is a lecithinase (phospholipase C)** that destroys cell membranes (myonecrosis, haemolysis), seen as the **Nagler reaction** (opalescence on egg-yolk agar neutralised by antitoxin) and "stormy fermentation" in litmus milk; **double zone of haemolysis** on blood agar; crepitus, bronze skin, thin brown foul discharge; treatment is surgical debridement, penicillin plus clindamycin, hyperbaric oxygen as adjunct; type A also causes food poisoning from the enterotoxin (reheated meat, cramps and watery diarrhoea in 8–24 hours).
- **Staphylococcus in bone and joint:** S. aureus is the commonest cause of osteomyelitis and septic arthritis in all ages (Salmonella characteristic in sickle cell disease, though S. aureus is still common there; Pseudomonas in puncture wounds through trainers, Kingella in young children, group B streptococcus in neonates); MRSA is mecA (PBP2a) mediated.
- **Mycobacterium leprae:** acid-fast with 5% sulphuric acid, **globi** in lepromatous disease; the Ridley–Jopling classification; **WHO multidrug therapy** (dapsone, rifampicin, clofazimine): classically two drugs (rifampicin, dapsone) for 6 months in paucibacillary disease, and since WHO 2018 the same three drugs for both, 6 months paucibacillary and 12 months multibacillary; single-dose rifampicin is WHO's post-exposure prophylaxis for contacts (2018); not cultivable in artificial media (grows in mouse footpad and armadillo); nasal secretions the main route.
- **Actinomyces israelii:** Gram-positive anaerobic branching filaments, **sulphur granules**, cervicofacial (lumpy jaw), pelvic (IUCD); penicillin; Nocardia is partially acid-fast and aerobic.
- **Brucella** and rickettsiae are bacterial zoonoses; **viridans streptococci** are the commonest cause of subacute native-valve endocarditis after dental work (S. gallolyticus bovis with colon cancer; Staphylococcus aureus in IV drug users and acute disease; coagulase-negative staphylococci on prosthetic valves). **HACEK organisms** (Haemophilus, Aggregatibacter, Cardiobacterium, Eikenella, Kingella) are slow-growing fastidious Gram-negative bacilli that cause culture-negative endocarditis; ceftriaxone. **Blood culture** (two or three sets from separate sites before antibiotics, 8–10 mL per bottle in adults, incubate 5 days) is essential in endocarditis and septicaemia; Salmonella Typhi is best isolated from blood in the first week, bone marrow culture is the most sensitive, stool and urine in the later weeks; Widal is unreliable.
- **Enterobacterales: Klebsiella pneumoniae**: capsule, mucoid lactose-fermenting colonies, non-motile, urease positive, citrate positive, red-currant jelly sputum, alcoholic and diabetic pneumonia, lung abscess, UTI, hypervirulent liver abscess; intrinsic ampicillin resistance, carbapenemases (KPC, NDM-1 common in South Asia). **Proteus mirabilis**: **swarming** on blood agar (suppressed on MacConkey), urease positive (alkaline urine and struvite stones), non-lactose fermenter, H_2S positive; Weil–Felix antigens: OX19 and OX2 are P. vulgaris strains, OXK is P. mirabilis. **Shigella**: non-motile, non-lactose fermenter, no H_2S, no gas; four species, S. dysenteriae 1 produces Shiga toxin; invades M cells; stool with pus cells and blood; ciprofloxacin or azithromycin; **no antimotility drugs**. **Enterococcus faecalis**: the Streptococcus faecalis of the curriculum, now reclassified as Enterococcus; bile-esculin positive, PYR positive, growth in 6.5% NaCl, UTI, endocarditis and biliary sepsis; ampicillin the first choice, vancomycin for VRE.
- **Laboratory diagnosis of urinary tract infection:** midstream clean-catch urine within 2 hours (or refrigerated, or in boric acid); **microscopy** with 10 or more pus cells per µL; **significant bacteriuria is 10^5 colony-forming units per mL (Kass criterion)** of a single organism on quantitative culture with a calibrated loop (0.001 mL), but a lower count (10^2–10^3) is significant in a symptomatic woman, with catheter samples or suprapubic aspiration (any growth); mixed growth of three or more types means contamination; culture on CLED or MacConkey and blood agar, then identify and test antibiotic sensitivity; nitrite and leucocyte esterase on dipstick; E. coli (uropathogenic with P fimbriae) causes 80% of community infections, S. saprophyticus in young women, Proteus with struvite stones, Pseudomonas and Enterococcus in hospital.

Virology

Classification and shapes

Group	Viruses	Notes
ssDNA	**Parvovirus B19** (also adeno-associated virus)	The only common human ssDNA viruses, small non-enveloped; aplastic crisis in haemolytic anaemia, erythema infectiosum, hydrops fetalis
dsDNA	Herpes (HSV, VZV, CMV, EBV, HHV-6, 7, 8), adeno, papilloma, polyoma, pox	HBV is a partly double-stranded circular DNA virus that replicates through an RNA intermediate (reverse transcriptase)

Group	Viruses	Notes
dsRNA	Rotavirus, reovirus	Segmented; rotavirus is the commonest cause of severe infant diarrhoea
+ssRNA	Picorna (polio, Hep A, rhino, coxsackie), flavi (dengue, Zika, JE, yellow fever, Hep C), toga (rubella), corona, calici, HIV (reverse transcribed)	Genome acts as mRNA
-ssRNA	Orthomyxo (influenza, segmented), paramyxo (measles, mumps, RSV), **rhabdo (rabies)**, filo (Ebola, Marburg), bunya (Hantavirus, CCHF), arena (Lassa)	Need their own polymerase; **rabies virus is bullet-shaped**

- Shapes: **rabies bullet**; **pox brick** (variola, molluscum); filovirus long filaments; Hepatitis B Dane particle 42 nm with 22 nm spheres and filaments of excess HBsAg; adenovirus icosahedral with fibres.
- Segmented genomes: influenza, rotavirus, bunyaviruses, arenaviruses, reoviruses; they allow reassortment.
- **Poxviruses** are the DNA viruses that replicate in the cytoplasm; other DNA viruses replicate in the nucleus. RNA viruses replicate in the cytoplasm except influenza, retroviruses and hepatitis D.
- Prions: abnormal protein (PrP-Sc), no nucleic acid; Creutzfeldt-Jakob disease, kuru, spongiform change.

Inclusion bodies

- **Negri bodies**: eosinophilic cytoplasmic inclusions of **rabies**, most abundant in **cerebellar Purkinje cells and hippocampal pyramidal neurons**; their detection confirms rabies. Fluorescent antibody test on a nuchal skin biopsy is the best antemortem test.
- Owl's eye (CMV, nuclear), Cowdry type A (herpes, nuclear), Guarnieri (variola, cytoplasmic), Molluscum bodies (Henderson-Paterson), Councilman bodies (apoptotic hepatocytes in yellow fever, hepatitis), Torres bodies (yellow fever), Reed-Sternberg (EBV association), smudge cells (adenovirus), Warthin-Finkeldey giant cells (measles), Koplik spots.

Hepatitis viruses

Virus	Family and route	Notes
HAV	Picorna, faeco-oral	Acute only, IgM anti-HAV; vaccine (killed or live)
HBV	Hepadna, blood, sexual, perinatal	Chronic in 90% of infected neonates, under 5% of adults
HCV	Flavi, blood	70-80% chronic; **direct-acting antivirals** (sofosbuvir-based) cure over 95% in 8-12 weeks
HDV	Defective, needs HBsAg	Coinfection or superinfection
HEV	Hepeviridae, faeco-oral	Genotype 1 is the epidemic type in Nepal; **mortality up to 20-25% in pregnant women**

Hepatitis B serology

- **HBsAg**: infection (acute or chronic; persisting over 6 months is chronic). **Anti-HBs**: immunity (vaccine alone, or after recovery with anti-HBc IgG). **HBeAg** reflects **active replication and high infectivity** (high HBV DNA). Anti-HBe suggests low replication. **Anti-HBc IgM**: acute or recent infection; the **only marker in the window period** between HBsAg disappearing and anti-HBs appearing. Anti-HBc IgG persists in all past exposure. HBV DNA is the most direct measure of replication; pre-core mutants are HBeAg negative with high DNA.
- Vaccine response: anti-HBs alone. Resolved infection: anti-HBs plus anti-HBc IgG. Chronic carrier: HBsAg plus anti-HBc IgG (IgM negative). Treatment of chronic HBV: tenofovir or entecavir (high barrier to resistance), or pegylated interferon in selected patients.
- Needle-stick risk after exposure: the exam's "rule of threes", HBV 30% (with an HBeAg-positive source; lower when the source is HBeAg negative), HCV 3%, HIV 0.3%; current CDC figures give HCV about 1.8%. Post-exposure prophylaxis for HBV: vaccine plus HBIG in non-immune.

HIV

- Retrovirus with **gp120 binding CD4 and a co-receptor: CCR5** (macrophage-tropic, early) **or CXCR4** (T-cell tropic, later); gp41 then mediates fusion. **CCR5 delta-32 homozygotes** are resistant. Reverse transcriptase, integrase, protease are enzyme targets. CD21 is the receptor for EBV; CD4 and CXCR4 for HIV.
- Diagnosis: fourth-generation antigen-antibody assay (p24 plus antibodies); rapid tests with confirmation by the national algorithm; HIV RNA for infants under 18 months (maternal IgG). Western blot is historical.
- Treatment: all patients on ART regardless of CD4 (WHO 2016 and later); first line dolutegravir with tenofovir and lamivudine or emtricitabine. Opportunistic infection prophylaxis: cotrimoxazole below CD4 200 (CDC; WHO advises it at CD4 350 or below, or WHO stage 3-4, in resource-limited settings) against PCP and toxoplasma, TB screening and preventive therapy, MAC below 50 in untreated. Cryptosporidium is a cause of chronic diarrhoea at low CD4; **nitazoxanide** and immune restoration.
- Mother to child: ART in pregnancy lowers transmission under 1%.

Dengue and arboviruses

- Dengue (flavivirus, four serotypes, Aedes aegypti and albopictus). Phases: febrile (2-7 days), **critical** at defervescence (plasma leakage, rising haematocrit, falling platelets, shock), recovery. **Warning signs** (WHO 2009): abdominal pain, persistent vomiting, mucosal bleeding, lethargy, liver enlargement, **haematocrit rise with rapid platelet fall**. Management: oral or intravenous **isotonic crystalloid with serial haematocrit** and vital signs; **avoid aspirin and NSAIDs**; no steroids; **platelet transfusion not for the count alone without bleeding**. NS1 antigen in the first 5 days, IgM from day 5. Vaccine: TAK-003 (Qdenga), two doses, recommended by WHO (position paper 2024) for children aged 6-16 in high-transmission settings. **Nepal**: dengue first recorded in 2004 and now endemic; large epidemics in 2019 (about 18,000 cases in 68 districts), 2022 (all 77 districts, Kathmandu the worst hit) and 2023 (about 50,000 cases by November, all 77 districts including the mountain districts) (WHO Disease Outbreak News 2022; Epidemiology and Disease Control Division reports).
- **Antibody-dependent enhancement**: severe dengue is more likely in a **second infection with a different serotype**, as non-neutralising antibody promotes Fc receptor uptake into monocytes.

- Japanese encephalitis: Culex (C. tritaeniorhynchus) breeding in rice fields; pigs amplify and ardeid birds (herons, egrets) are the reservoir; man is a dead-end host; endemic in the Nepali terai and increasingly the hills; vaccine at 12 months in Nepal's national programme. Chikungunya: arthralgia. Zika: microcephaly. Yellow fever: Torres bodies; 17D vaccine.

Rabies

- Bullet-shaped rhabdovirus; travels retrogradely along nerves; hydrophobia, aerophobia, furious or paralytic forms; almost always fatal. Wound washing with soap and water for 15 minutes; **rabies immunoglobulin infiltrated into the wound** in category III; vaccine intradermal short-course schedules (WHO 2018) or intramuscular; pre-exposure vaccination for high-risk workers.

Influenza and coronaviruses

- Influenza A: **antigenic shift** (reassortment of segments, e.g. avian-human, pandemics) versus **antigenic drift** (point mutations in haemagglutinin and neuraminidase, seasonal epidemics). Oseltamivir, ideally within 48 hours; baloxavir. Type B has no animal reservoir and only drifts.
- SARS-CoV-2: positive-sense RNA virus; RT-PCR; BSL-2 for diagnostic sample handling, **BSL-3 for culture**. Variants mainly in spike; nirmatrelvir-ritonavir, remdesivir, dexamethasone in hypoxaemia.

Other points

- Herpes: HSV-1 oral (temporal lobe encephalitis, acyclovir), HSV-2 genital; VZV chickenpox and zoster (dorsal root ganglia); CMV (owl's eye, retinitis and colitis in AIDS, ganciclovir); EBV (infectious mononucleosis, heterophile Paul-Bunnell, Burkitt, nasopharyngeal carcinoma). HHV-8: Kaposi sarcoma.
- **EBV versus CMV mononucleosis**: both give fever, lymphocytosis and atypical lymphocytes; EBV is heterophile (monospot) positive with prominent exudative pharyngitis and lymphadenopathy; **CMV mononucleosis is heterophile negative**, with little sore throat (the commonest cause of monospot-negative mononucleosis).
- **Roseola infantum (exanthem subitum, sixth disease)**: **HHV-6** (mostly 6B; sometimes HHV-7) in infants of 6 months to 2 years; three to five days of high fever, then a rose-pink rash on the trunk as the fever falls; febrile seizures are common.
- **Parvovirus B19** (ssDNA, non-enveloped): binds the **P antigen (globoside)** on erythroid progenitors and kills them, stopping red cell production for about a week: erythema infectiosum (slapped cheek, fifth disease), **transient aplastic crisis** in shortened red cell survival (sickle cell disease, hereditary spherocytosis), chronic anaemia in the immunocompromised, **hydrops fetalis** in pregnancy, symmetrical arthropathy in adult women.
- **Polyomaviruses** (dsDNA): **JC virus** causes progressive multifocal leukoencephalopathy; **BK virus** causes **nephropathy in kidney transplants** (decoy cells in urine, enlarged tubular nuclei with inclusions staining for SV40 large T antigen, plasma BK PCR for screening; treatment is reducing immunosuppression) and haemorrhagic cystitis after marrow transplant.
- HPV: 16 and 18 cervical cancer (E6 degrades p53, E7 binds Rb); 6 and 11 warts. Polio: faeco-oral, anterior horn cells, vaccine-derived strains. Measles: Koplik spots, SSPE. Mumps: parotitis, orchitis. Rubella: congenital rubella (cataract, patent ductus, deafness).
- Antivirals: acyclovir (viral thymidine kinase), ganciclovir (CMV), foscarnet (pyrophosphate analogue; used in resistant CMV), ribavirin, oseltamivir.

Viruses named in the MBBS curriculum: facts to revise

- **Poliovirus:** picornavirus (non-enveloped ssRNA+, three serotypes); faeco-oral; replicates in gut lymphoid tissue, then viraemia and **anterior horn cell** destruction: asymmetric acute flaccid paralysis, no sensory loss; most infections are inapparent (about 1% paralytic). Diagnosis: stool virus isolation in cell culture (two stool samples 24–48 hours apart, within 14 days of onset) and sequencing, AFP surveillance, CSF with mild pleocytosis; no antiviral. **OPV** (Sabin, live attenuated, gives gut immunity and herd protection but rare vaccine-associated paralysis and vaccine-derived strains) and **IPV** (Salk, killed, no gut immunity but safe); wild type 2 and 3 are eradicated; Nepal and India are certified polio-free by WHO (SEARO 2014).
- **Rabies virus:** bullet-shaped rhabdovirus (ssRNA−, enveloped) travelling by retrograde axonal transport to the CNS; **Negri bodies** (cytoplasmic eosinophilic inclusions, hippocampus and Purkinje cells); incubation 1–3 months (varies); hydrophobia and aerophobia; diagnosis by skin biopsy nuchal direct fluorescent antibody and RT-PCR; wound washing, rabies immunoglobulin infiltrated into the wound and cell-culture vaccine (WHO 2018 categories I–III; the intradermal one-week schedule is a WHO option). Dogs cause up to 99% of human cases (WHO).
- **Japanese encephalitis virus:** flavivirus; **Culex tritaeniorhynchus** in rice fields; **pigs are the amplifying hosts** (high viraemia, no illness), water birds (herons, egrets) the natural reservoir, humans and horses dead-end hosts; children get encephalitis with parkinsonian features; diagnosis by **IgM capture ELISA in CSF or serum**; vaccination with live attenuated SA 14-14-2, one dose at 12 months in Nepal's routine schedule (the programme began in the endemic terai districts).
- **Adenoviruses:** non-enveloped dsDNA icosahedral with fibres; pharyngoconjunctival fever, **haemorrhagic cystitis (types 11, 21)**, epidemic keratoconjunctivitis, infant gastroenteritis (types 40 and 41), pneumonia; basophilic intranuclear inclusion ("smudge" cells); cytopathic effect of grape-like clusters in cell culture; diagnosis by PCR and antigen detection.
- **Myxoviruses:** orthomyxoviruses (influenza A, B, C: segmented ssRNA−, haemagglutinin and neuraminidase) and **paramyxoviruses** (measles, mumps, RSV, parainfluenza: non-segmented; **fusion protein; syncytia**); parainfluenza causes croup (steeple sign), RSV bronchiolitis (palivizumab, nirsevimab); measles has Koplik spots and Warthin–Finkeldey giant cells.
- **Rhinoviruses:** picornaviruses with over 100 serotypes, acid-labile (so they do not survive in the stomach), grow best at 33 °C, cause the common cold; spread by hands and aerosol; no vaccine possible because of the serotypes.
- **SARS (2003) coronavirus:** emerged in Guangdong in 2002–03, **Carlo Urbani** (a WHO doctor from Italy) first recognised the disease in Hanoi in February 2003 and died of it; the reference strain is named Urbani after him; civet cats the likely intermediate host and bats the reservoir; atypical

pneumonia, spread by droplets; ACE2 receptor; about 10% case fatality; no cases since 2004. MERS is camel-associated.

- **Avian influenza (H5N1, H7N9):** influenza A in wild birds and poultry; humans are infected by close contact with infected poultry; severe pneumonia with high case fatality; limited human-to-human spread; reassortment with human strains is the pandemic fear; oseltamivir. Since 2021 H5N1 clade 2.3.4.4b has spread across wild birds and mammals worldwide, and from 2024 in dairy cattle in the United States with mild human infections (conjunctivitis) among farm workers (WHO, CDC 2024–25).
- **Rotavirus** (dsRNA, 11 segments, wheel shape; commonest cause of severe infant diarrhoea; oral live vaccines; ELISA antigen in stool); **Norwalk (norovirus)**: calicivirus, ssRNA+, winter vomiting disease, cruise-ship and food-handler outbreaks, short incubation (12–48 hours), diagnosis by RT-PCR, no vaccine.
- **Herpes simplex virus 2 (genital herpes) and CMV:** HSV-2 establishes latency in the **sacral ganglia**, causes recurrent painful vesicles and neonatal herpes at delivery (caesarean if lesions); diagnosis by PCR of lesion fluid (replacing Tzanck smear showing multinucleated giant cells); aciclovir. **Cytomegalovirus** (human herpesvirus 5): latent in monocytes, "owl's-eye" intranuclear inclusion, commonest congenital infection (hearing loss, periventricular calcification), retinitis and colitis in AIDS (ganciclovir, foscarnet), mononucleosis-like illness, post-transplant; diagnosis by PCR, pp65 antigenaemia, IgM and IgG avidity.
- **Oncogenic viruses:** HPV 16 and 18 (cervix, anus, oropharynx), EBV (Burkitt lymphoma, nasopharyngeal carcinoma, Hodgkin and post-transplant lymphomas), HBV and HCV (hepatocellular carcinoma), HTLV-1 (adult T-cell leukaemia), HHV-8 (Kaposi sarcoma), Merkel cell polyomavirus. Mechanisms: viral oncoproteins that inactivate p53 and Rb (HPV E6 and E7), a transactivating viral protein (HTLV-1 Tax, which switches on host growth genes; insertional activation is the mechanism of slow-transforming animal retroviruses), chronic inflammation and cirrhosis (HBV and HCV).

Mycology

Basics

- Yeasts reproduce by budding; moulds by hyphae. **Thermally dimorphic fungi**: mould at 25°C, yeast at 37°C: **Sporothrix schenckii**, Histoplasma, Blastomyces, Coccidioides, Paracoccidioides, Talaromyces (Penicillium) marneffei. **Candida and Cryptococcus are yeasts** in both settings; Candida also forms pseudohyphae; Aspergillus and Mucorales are moulds throughout.
- Wet mounts: 10-20% **KOH** dissolves keratin; calcofluor white fluorescence; Gomori methenamine silver and PAS in tissue; Sabouraud dextrose agar for culture (room temperature, 4 weeks).

Superficial and cutaneous

- **Malassezia furfur**: pityriasis (tinea) versicolor, hypo- or hyperpigmented patches, KOH shows **"spaghetti and meatballs"** (short curved hyphae and round yeast cells); also seborrhoeic dermatitis and folliculitis; yellow-green fluorescence under Wood's light; treated with topical selenium sulphide or azoles. Lipophilic yeast, needs olive oil on culture.
- Dermatophytes: Trichophyton, Microsporum, Epidermophyton; septate hyphae in KOH; tinea capitis (griseofulvin or terbinafine), tinea corporis, tinea pedis, onychomycosis (terbinafine). Microsporum fluoresces with Wood's light.
- Candida: oral thrush, vulvovaginitis, oesophagitis (AIDS-defining), candidaemia; germ tube test positive for C. albicans (yeast cells in serum at 37°C for 2 to 3 hours put out germ tubes with no constriction at the origin; C. dubliniensis too, C. glabrata negative; also chlamydospores on cornmeal agar). C. auris: multidrug-resistant, hospital outbreaks. First line for candidaemia: echinocandin (IDSA 2016, still current).

Subcutaneous and systemic

- **Sporothrix**: rose gardener's disease, lymphocutaneous spread, cigar-shaped yeasts; itraconazole, or potassium iodide.
- Mycetoma (Madura foot): fungal (eumycetoma) or actinomycetoma, grains in sinuses. Chromoblastomycosis: sclerotic bodies.
- **Rhinosporidium seeberi**: friable, pink, strawberry-like polypoid nasal (also conjunctival) masses studded with white dots (sporangia), after bathing in stagnant ponds; common in South Asia (India and Sri Lanka most, reported from Nepal). Tissue or KOH shows **large sporangia (up to 300 µm) full of endospores**; it **cannot be cultured**; treatment is surgical excision with cautery of the base (dapsone as an adjunct against recurrence). Taught with the fungi, but now classed as an aquatic protist (Mesomycetozoea), not a true fungus.
- **Cryptococcus neoformans**: encapsulated yeast; **India ink** halo in CSF; **cryptococcal antigen** (lateral flow, serum or CSF) is more sensitive; C. neoformans from pigeon droppings, C. gattii from Eucalyptus trees; meningitis in AIDS: amphotericin B plus flucytosine induction, then fluconazole; control raised pressure with serial lumbar punctures. **Exam answer**: amphotericin B plus flucytosine induction. **Current practice** (WHO 2022): a single 10 mg/kg dose of liposomal amphotericin B with 14 days of flucytosine and fluconazole; screening for serum cryptococcal antigen in advanced HIV (CD4 below 100) with pre-emptive fluconazole.
- **Aspergillus**: narrow septate hyphae, **acute-angle (45°) branching**; allergic bronchopulmonary aspergillosis, aspergilloma, invasive disease in neutropenia (voriconazole first line, galactomannan test); aflatoxin causes hepatocellular carcinoma.
- **Mucormycosis (Mucorales: Rhizopus, Mucor, Rhizomucor)**: **broad, ribbon-like, pauciseptate hyphae with right-angle branching**; rhino-orbito-cerebral form in **ketoacidosis** and iron overload; black eschar, proptosis; surgical debridement plus liposomal amphotericin B; posaconazole or isavuconazole step-down; surge in COVID-19-associated disease in South Asia (diabetes and steroids).
- Histoplasma: intracellular yeasts in macrophages, bird and bat droppings; Blastomyces: broad-based budding; Coccidioides: spherules with endospores; Pneumocystis jirovecii: ground-glass pneumonia, cotrimoxazole first line, steroids if hypoxic, Gomori silver and beta-D-glucan.
- Antifungals: amphotericin B (ergosterol binding, nephrotoxicity), azoles (CYP51, ergosterol synthesis), echinocandins (beta-glucan synthase; not active against Cryptococcus), terbinafine (squalene epoxidase), griseofulvin (microtubule), flucytosine (5-FU conversion).

Fungi named in the MBBS curriculum: practical points

- **Specimen handling: KOH 10–20% wet mount** (dissolves keratin, shows hyphae of dermatophytes, pseudohyphae and yeast cells of Candida); **lactophenol cotton blue** for slide culture and moulds (cotton blue stains chitin, lactic acid clears, phenol kills); **India ink** for capsules (Cryptococcus); **Sabouraud dextrose agar** (pH 5.6, with chloramphenicol; cycloheximide for dermatophytes) at 25–30 °C for up to 4 weeks, with duplicate tubes at 37 °C for dimorphism; **germ tube test** (serum at 37 °C for 2–3 hours: C. albicans); calcofluor white fluorescence; periodic acid–Schiff and Gomori methenamine silver in tissue; Wood's lamp for tinea capitis (Microsporum green).
- **Dermatophytes:** Trichophyton (skin, hair, nails; T. rubrum commonest, T. tonsurans in endothrix scalp infection), **Microsporum** (skin and hair, not nails; M. canis; macroconidia spindle-shaped), **Epidermophyton floccosum** (skin and nails, not hair; club-shaped smooth macroconidia). Keratinophilic; diagnosis by KOH and culture; terbinafine or griseofulvin (tinea capitis; griseofulvin acts on microtubules and needs a fatty meal), itraconazole or topical azoles. **Trichophyton indotineae** (epidemic in South Asia including Nepal through the 2010s, named in 2020, now reported worldwide) causes extensive, recurrent tinea corporis and cruris, often resistant to terbinafine (squalene epoxidase mutations), fuelled by topical steroid–antifungal creams; itraconazole for several weeks is the usual treatment (Indian Association of Dermatologists' consensus 2018; WHO and CDC reports 2023).
- **Aspergillus fumigatus:** septate hyphae branching at **acute (45°) angles**; sources: soil and decaying vegetation; allergic bronchopulmonary aspergillosis (asthma, eosinophilia, high IgE), **aspergilloma** (fungus ball in an old tuberculous cavity), invasive aspergillosis in neutropenia (halo sign on CT, serum galactomannan, beta-D-glucan, voriconazole first choice), aflatoxin from Aspergillus flavus (hepatocellular carcinoma). Mucor has broad aseptate hyphae with right-angle branching.
- **Histoplasma capsulatum:** thermally dimorphic, small intracellular yeasts in macrophages, tuberculate macroconidia in the mould form; bat and bird droppings in caves (Ohio and Mississippi valleys, also reported elsewhere); resembles tuberculosis in the lung; urinary antigen; itraconazole or amphotericin B.
- **Pneumocystis jirovecii** (formerly carinii; classified now as a fungus; cannot be cultured): **Gomori methenamine silver stains the cyst wall** (cup, crushed ping-pong ball or helmet-shaped cysts, 4–8 µm); **Giemsa or Wright** stains trophozoites and intracystic bodies but not the wall; direct fluorescent antibody and PCR are more sensitive; sample: **bronchoalveolar lavage** or induced sputum; raised serum LDH and beta-D-glucan; bilateral ground-glass opacities; AIDS with CD4 below 200; treat with high-dose cotrimoxazole plus steroids for hypoxaemia (PaO_2 below 70 mmHg); prophylaxis with cotrimoxazole.

Parasitology

Host and transmission terms

- **Definitive host**: harbours the adult or sexual stage. In **malaria the mosquito is the definitive host and man the intermediate host**; Leishmania has no sexual stage, and by convention man (the vertebrate) is called its definitive host and the sandfly its intermediate host. Intermediate host: harbours larval or asexual stages. Reservoir; paratenic host.
- Arthropod-borne transmission: **cyclo-propagative** (the agent changes form and multiplies: **malaria in Anopheles**, trypanosomes); cyclo-developmental (changes form but does not multiply: **filariae**); propagative (multiplies without change: plague bacillus in fleas, dengue); mechanical (carried only).
- Snail hosts: **Bulinus** (Schistosoma haematobium), Biomphalaria (S. mansoni), Oncomelania (S. japonicum), Lymnaea (Fasciola), Planorbis; Fasciola also needs aquatic plants for the metacercariae.

Protozoa: intestinal and tissue

- **Entamoeba histolytica**: cyst (infective, quadrinucleate) and trophozoite with ingested red cells; flask-shaped ulcers, amoebic liver abscess ("anchovy sauce" pus), treated with metronidazole followed by a luminal agent (paromomycin or diloxanide). Giardia lamblia: pear-shaped trophozoite, falling-leaf motility, cyst in stool, steatorrhoea; tinidazole or metronidazole.
- **Cryptosporidium**: small (4-6 µm) **acid-fast oocysts** on modified Ziehl-Neelsen; profuse watery diarrhoea in advanced HIV; **nitazoxanide** plus ART. Cyclospora (larger, 8-10 µm, acid-fast, autofluorescent; cotrimoxazole), Isospora (cotrimoxazole).
- Toxoplasma: cat definitive host; congenital (chorioretinitis, hydrocephalus, calcifications) and AIDS encephalitis (ring-enhancing lesions; pyrimethamine plus sulphadiazine).
- **Balantidium coli**: the **only ciliate** and the **largest protozoan** parasite of humans; pigs are the reservoir; trophozoites invade the colon, causing ulcers and dysentery like amoebiasis; tetracycline (metronidazole an alternative).
- Naegleria fowleri: amoebic meningoencephalitis after warm fresh water, nasal route; Acanthamoeba: keratitis in lens wearers.
- Trichomonas vaginalis: motile flagellate, strawberry cervix, metronidazole treating the partner.

Leishmaniasis

- Amastigotes (LD bodies) in macrophages of the reticuloendothelial system; promastigote is the form in the sandfly and culture (NNN medium); the amastigote is the form in humans.
- **Visceral leishmaniasis (kala-azar)**: *L. donovani*; vector **Phlebotomus argentipes** in the Indian subcontinent (P. papatasi cutaneous in the Old World; Lutzomyia in the Americas). Fever, massive splenomegaly, pancytopenia, hypergammaglobulinaemia, skin darkening. **rK39 immunochromatographic strip** is the rapid field test; confirm by splenic, bone marrow or lymph node aspirate; the formol-gel test is historical. **Post-kala-azar dermal leishmaniasis (PKDL)**: macules, papules or nodules, in South Asia usually 2 to 3 years after treated visceral disease (in Sudan during or within months of it); the lesions carry parasites that infect sandflies, so patients are an **infectious reservoir between epidemics**, and finding and treating them is part of the elimination programme (in South Asia oral miltefosine for 12 weeks is the usual treatment, liposomal amphotericin B the alternative). **Nepal**: historically endemic in the eastern and central terai (about 18 districts by the endemic definition), but cases are now reported from about 50 districts, including western and hill districts (Kalikot, Dailekh, Palpa, Pyuthan) and in children; the elimination target, under the South-East

Asia regional programme since 2005, is **fewer than 1 case per 10,000 population at district level in Nepal** (sub-district or block level in India and Bangladesh); Nepal first reached it in 2013 and is seeking WHO validation (WHO; national reports 2025-2026).

- **Treatment in South Asia: a single dose of liposomal amphotericin B 10 mg/kg** (WHO-recommended first line for the subcontinent); alternatives are multi-dose AmBisome, miltefosine (teratogenic) combinations, paromomycin; antimonials are no longer used because of resistance in Bihar.

Malaria

- Plasmodium falciparum (malignant tertian; banana gametocytes; multiple rings in red cells of all ages; cerebral malaria and blackwater fever; **knobs on red cells cause sequestration**), vivax and ovale (tertian, 48-hour cycle, **hypnozoites** in the liver), malariae (quartan 72 hours, nephrotic syndrome), knowlesi (24-hour cycle, zoonotic).
- **P. vivax**: enlarged red cells with **Schüffner's dots**; the **Duffy antigen (receptor for chemokines)** is the **receptor for merozoite invasion**, so Duffy-negative people are resistant (West Africa); falciparum uses glycophorins. Sickle trait and G6PD deficiency also protect, as does Hb F.
- Diagnosis: thick film (detection), thin film (species), rapid antigen tests (HRP2 for falciparum, pLDH for others); HRP2 deletions are reported.
- Treatment: artemisinin-based combination therapy for falciparum (WHO 2022: artemether-lumefantrine, artesunate-amodiaquine, others); severe malaria: IV artesunate; vivax: chloroquine where sensitive (or ACT) plus **primaquine for radical cure** of the hypnozoites (14 days) **after G6PD testing** (haemolysis), tafenoquine single dose is an alternative; pregnancy: no primaquine. Chemoprophylaxis: doxycycline, atovaquone-proguanil, mefloquine. **Nepal**: P. vivax causes most cases, with P. falciparum rising; most cases are now imported (largely across the open border with India), the indigenous ones mainly in the far-western terai (Kailali, Kanchanpur) with some now in hill and mountain districts; the elimination target was moved from 2026 to 2030 (Epidemiology and Disease Control Division, reported 2024-2025). Nepal treats vivax with chloroquine and 14 days of primaquine.
- Lifecycle: sporozoites to the liver; erythrocytic schizogony; gametocytes taken by female Anopheles; sexual stage and sporogony in the mosquito. Transplacental and transfusion malaria bypass the liver stage, so no hypnozoite relapse.

Other protozoa

- Trypanosomiasis: African (tsetse; T. brucei gambiense and rhodesiense; sleeping sickness); American (Chagas disease, Trypanosoma cruzi, reduviid bug, megacolon and cardiomyopathy). Babesia: Maltese cross in red cells, Ixodes ticks.

Nematodes

- **Ascaris lumbricoides**: largest roundworm; **embryonated egg** is the infective form (swallowed from soil-contaminated food); larvae hatch, migrate through liver and lung (Loeffler pneumonia) and mature in the small intestine. Fertilised eggs have a mammillated coat. Albendazole or mebendazole single dose. Complications: intestinal obstruction, biliary ascariasis.
- Hookworms (Ancylostoma, Necator): **filariform larva infects through skin** (barefoot), causes iron-deficiency anaemia; albendazole. Trichuris trichiura: barrel-shaped eggs, rectal prolapse. Enterobius vermicularis (pinworm): perianal itch, Scotch tape test, no tissue stage.
- **Strongyloides stercoralis**: rhabditiform larvae in stool; the filariform larva penetrates skin or the gut wall (**autoinfection**); **hyperinfection with corticosteroids or immunosuppression** (HTLV-1) with Gram-negative sepsis; ivermectin treats it. Steroids should not be started without treating a possible strongyloides in endemic areas.
- Filaria: Wuchereria bancrofti (nocturnal periodicity, culex; elephantiasis, hydrocele; mf detected in night blood, DEC plus albendazole in mass drug administration; WHO since 2017 also advises the triple IDA regimen, ivermectin, DEC and albendazole, where onchocerciasis and loiasis are absent), Brugia malayi (Mansonia). Loa loa (eyeworm), Onchocerca volvulus (river blindness, ivermectin), Dracunculus. Trichinella: undercooked pork, myositis and periorbital oedema, eosinophilia.

Cestodes and trematodes

- **Taenia solium (pork tapeworm)**: eating **undercooked pork** gives **intestinal taeniasis**; **swallowing eggs** (faeco-oral, from a human carrier, food, hands) gives **cysticercosis** (larvae in brain, muscle, eye). Neurocysticercosis: commonest cause of acquired epilepsy in endemic countries, including Nepal; CT shows a cyst with a **dot-like scolex** ("hole-with-dot"); albendazole plus steroids, with anticonvulsants; avoid anthelmintic in lesions that are only calcified. T. solium scolex has a rostellum with hooks; T. saginata (beef) has no hooks and does not cause cysticercosis. Taenia eggs are identical: the species is told from the proglottid uterine branches (T. saginata 15 or more on each side, about 15-30; T. solium fewer than 13, about 7-13; texts vary) and the scolex. Praziquantel treats the adult worm.
- Echinococcus granulosus: dog definitive host, sheep intermediate; hydatid cyst in liver and lung; do not aspirate (anaphylaxis); PAIR or surgery with albendazole; Casoni test historical. Diphyllobothrium: vitamin B12 deficiency from fish. Hymenolepis nana: the only cestode needing no intermediate host.
- **Schistosoma haematobium**: terminal-spined eggs, **terminal haematuria**, bladder wall calcification, squamous carcinoma of the bladder; intermediate host **Bulinus**; treated with praziquantel. S. mansoni (lateral spine, Biomphalaria; hepatosplenic), S. japonicum (small spine, Oncomelania). Cercariae penetrate skin.
- Fasciola hepatica: liver fluke, sheep liver rot, shoulder spine; triclabendazole. Clonorchis, Opisthorchis: cholangiocarcinoma; praziquantel. Paragonimus: lung fluke, "rusty" sputum, crabs.

Diagnostic reminders

- Eosinophilia is typical of tissue helminths (Strongyloides, Ascaris larval phase, filaria, Trichinella, Toxocara) and not of Giardia or established intestinal cestodes. Stool concentration with formol-ether; Kato-Katz for egg counts.
- Larva migrans: cutaneous (Ancylostoma braziliense, creeping eruption), visceral (Toxocara canis).
- Antiparasitic drugs: albendazole (tubulin), ivermectin (glutamate chloride channels), praziquantel (calcium influx), DEC (filaria), metronidazole and tinidazole (anaerobic protozoa), artemisinins, chloroquine, primaquine, pyrimethamine.

Parasites named in the MBBS curriculum: facts to revise

- **Entamoeba histolytica: trophozoites containing ingested red blood cells (erythrophagocytosis)** indicate invasive disease and are not seen in the non-pathogenic E. dispar and E. moshkovskii (these cannot be told apart by microscopy; PCR, antigen tests and zymodeme analysis distinguish them). Quadrinucleate cysts (chromatoid bars) are in formed stool; hot (fresh) stool for motile trophozoites with active, directional movement by a single pseudopodium (E. coli moves sluggishly); culture in Robinson medium; serology is positive in liver abscess. Ulcers are flask-shaped; treatment: metronidazole or tinidazole, then paromomycin or diloxanide.
- **Giardia lamblia:** pear-shaped trophozoite with two nuclei (monkey face), sucking disc, falling-leaf motility; oval cyst with four nuclei; drinking contaminated water (streams, untreated supplies; travellers) or person-to-person spread; fatty, foul, bulky stool without blood, malabsorption; stool antigen or PCR is the best; **string test or duodenal aspirate** if stool is negative; metronidazole, tinidazole or nitazoxanide.
- **Coccidian parasites:** Cryptosporidium (acid-fast oocysts 4–6 µm), Cyclospora (oocysts 8–10 µm, autofluorescent), Cystoisospora belli (large oval oocyst; unlike most protozoa it can cause eosinophilia): watery diarrhoea in AIDS; modified Ziehl–Neelsen staining; nitazoxanide, cotrimoxazole.
- **Toxoplasma gondii:** obligate intracellular; **cat the definitive host** (oocysts in faeces), intermediate hosts humans, sheep, pigs; infection by undercooked meat, cat faeces; tachyzoites in acute infection, bradyzoites in tissue cysts; the **classic congenital triad** is chorioretinitis, hydrocephalus and intracranial calcification; reactivation in AIDS gives ring-enhancing brain lesions; diagnosis by IgM, IgG avidity, PCR; spiramycin in pregnancy, pyrimethamine plus sulfadiazine with folinic acid.
- **Intestinal nematodes:** Ascaris (fertilised and unfertilised eggs, mammillated coat; Loeffler pneumonitis; albendazole); **hookworm** (Ancylostoma duodenale: two pairs of teeth; Necator americanus: cutting plates; filariform larva penetrates skin; eggs thin-shelled with four to eight cells in fresh stool; iron deficiency anaemia from chronic blood loss (about 0.03 mL a day per Necator worm, 0.15–0.25 mL per Ancylostoma duodenale worm); albendazole); **Enterobius** (pinworm: perianal itching at night, **cellophane (Scotch) tape test or NIH swab** in the morning; asymmetric eggs with a flattened side; family treatment); **Trichuris** (whipworm: lemon- or barrel-shaped eggs with polar plugs; rectal prolapse in heavy infection; mebendazole or albendazole).
- **Cestodes:** Taenia solium (scolex with hooks and four suckers; gravid proglottid with fewer than 13 lateral branches; cysticercosis by eggs, brain cysts; neurocysticercosis is a leading cause of acquired epilepsy in Nepal and other endemic countries), T. saginata (no hooks, over 13 branches); **Echinococcus granulosus** (dog is the definitive host, humans intermediate hosts, hydatid cyst with the double wall and daughter cysts, hydatid sand; Casoni test historical, serology and imaging now; albendazole with PAIR or surgery); **Hymenolepis nana (dwarf tapeworm)**: the smallest human tapeworm (2–4 cm), no intermediate host is required (direct autoinfection, also grain beetles), eggs with polar filaments in a clear shell, auto-infection causes heavy burden; **praziquantel** drug of choice.
- **Plasmodium species and the blood film:** thin film for species, thick film for detection (20–40 times more sensitive); stain with Giemsa or Leishman at pH 7.2; rapid antigen tests (HRP-2 for P. falciparum); **Nepal's malaria is mostly P. vivax**, with falciparum in the terai; chloroquine for vivax (plus primaquine 14 days, after G6PD testing; tafenoquine single-dose option), artemisinin combination therapy (artemether-lumefantrine) for falciparum, injectable artesunate for severe malaria; chemoprophylaxis with atovaquone-proguanil, doxycycline, mefloquine.
- **Wuchereria bancrofti:** the adult worms live in lymphatics; the **microfilariae show nocturnal periodicity** in peripheral blood (peak 10 pm to 2 am), matched to the biting time of the vector (Culex quinquefasciatus), so a blood film is collected **around midnight**; sheathed microfilariae with nuclei not reaching the tail tip; concentration by Knott's technique or membrane filtration; the **immunochromatographic card test** (antigen) works in the daytime; diurnal and subperiodic forms exist in the Pacific; treated with DEC plus albendazole (WHO triple therapy with ivermectin, IDA); elephantiasis, chyluria, hydrocele.
- **Leishmania donovani:** amastigotes (LD bodies) in macrophages in bone marrow, spleen or lymph node smears with Giemsa; promastigotes in NNN medium; **rK39 rapid test** (serology; the standard field test in South Asia); sandfly Phlebotomus argentipes; liposomal amphotericin B (single dose) is the current first-line in South Asia; miltefosine and paromomycin are alternatives; post-kala-azar dermal leishmaniasis; the South Asian elimination target is below 1 case per 10,000 population a year (district level in Nepal, sub-district or block level in India and Bangladesh).
- **Paragonimus westermani:** lung fluke, acquired by eating undercooked freshwater crabs or crayfish, golden-brown operculated eggs in **rusty sputum**; praziquantel.

Sources

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