

Staphylococcus Aureus

- S. aureus produces an enzyme coagulase (basis of coagulase test)
- Coagulase negative staphylococci (CONS) are rarely pathogenic to man eg: S. epidermidis, S. saprophyticus, S. lugdunensis, S. schleiferi, S. haemolyticus, S. warneri
- 'Staphyle' means berry-shaped
- S. aureus was so named based on the characteristic golden yellow pigmentation of its colonies on nutrient agar
- S. aureus - catalase positive
 - coagulase positive
 - β hemolytic colonies on blood agar

Virulence Factors:

Cell Wall Associated Factors:

- Peptidoglycan
- Teichoic acid
- Cell surface adhesins
- Protein A

Toxins:

- Membrane active toxins:
 - ⇒ hemolysins $\alpha, \beta, \delta, \epsilon$
 - ⇒ leukocidin (or panton valentine toxin)
- Epidermolytic toxin (exfoliative toxin)
- Enterotoxins
- Toxic shock syndrome toxin

Extracellular Enzymes:

- Coagulase (prothrombin-like)
- Heat stable thermonuclease
- Deoxyribonuclease
- Staphylokinase (fibrinolysin)
- Hyaluronidase, lipase, protease

(CspA) →

- **Protein-A:** is anti-complementary, chemotactic, mitogenic, inhibition of opsonization, induction of platelet damage
- **Hemolysins:** act on RBCs leading to hemolysis (actions - dehemecrotic, cytotoxic, neurotoxic, leukocidal)
- **Leukocidins / Pantone Valentine Toxin:** (PV toxin) it acts synergistically with δ -hemolysin to damage leukocytes, RBCs & macrophages
- **Synergohemolytic toxins:** δ -hemolysin & PV toxin are called synergohemolytic toxins because they are not active individually, their combination has hemolytic & leukocidal activity. [PV toxin is expressed on MRSA]
- **Epidermolytic/Exfoliative Toxin:** it is responsible for staphylococcal scalded skin syndrome [SSSS]
 - severe form in infants is called Ritter's syndrome: fever, lethargy, irritability with poor feeding
 - milder form: pemphigus neonatorum & bullous impetigo.
- **Enterotoxins:** cause staphylococcal food poisoning
- **Toxic Shock Syndrome Toxin (TSST):**
 - two subtypes - TSST 1 & TSST 2
 - **Risk factors:** using highly absorbent vaginal tampons during menstruation, complication of staphylococcal abscesses, osteomyelitis, wounds

- **Pathogenesis:** TSST 1 gets absorbed into circulation from tampons being a superantigen, it stimulates T-cells non-specifically
 - ↓
 - cytokine storm
 - ↓
 - potentially fatal multisystem disease

- **Clinical features:** fever, hypotension, diarrhoea, erythematous rashes
- **Clinical Criteria for TSS:** *fever *rash *desquamation *hypotension *multiorgan (≥ 3) involvement
- **Laboratory Criteria for TSS:** negative blood culture for other pathogens
- Anti-TSST antibodies usually appear in the convalescent stage & are protective
- **Diagnosis:** latex agglutination test & enzyme immunoassay, PCR-based assays for gene detection
- **Treatment:** TSST causes capillary leak $\therefore$ aggressive parenteral fluid replacement & vasopressor to reverse hypotension
- * Clindamycin is the preferred drug for TSST.

- **Coagulase:** principle virulence factor of S. aureus
 - brings about clotting by activating prothrombin
 - fibrin clot surrounding the bacteria protects it from phagocytosis & other defense mechanisms
- Heat stable thermonuclease & DNase are specific to S. aureus
- Staphylokinase (fibrinolysin) breaks down fibrin clots to facilitate the spread of infection.
- Hyaluronidase breaks down connective tissue

Pathogenesis of S. aureus:

- **Colonization:** S. aureus colonizes on various body surfaces
- **Introduction into tissue:** as a result of minor abrasions
 - they then adhere to tissue surfaces mediated by adhesins (clumping factor & collagen-binding adhesin)
- **Invasion:** into tissues by serine proteases, hyaluronidases, thermonucleases & lipases which facilitate local spread
- **Evasion of host defense mechanisms:** such as anti-phagocytic activity, inhibition of leukocyte migration, intracellular survival inside endothelial cells
- **Metastatic spread:** by hematogenous route

Clinical Manifestations: S. aureus causes both healthcare & community-associated infections ranging from minor skin & soft tissue infections to life-threatening systemic infections.

- **Epidemiology:** most common sites of colonization - anterior nares & oropharynx, abraded skin, vagina, axilla, perineum.
- S. aureus is a part of normal human flora
- rate of colonization is higher in the immunocompromised.

Laboratory Diagnosis:

- **Samples:** - for carriers $\Rightarrow$ nasal swabs
- for blood culture $\Rightarrow$ automated culture bottles
- for food poisoning $\Rightarrow$ feces, vomitus, food

Direct Smear Microscopy: Colony morphology as observed -

- **Nutrient Agar:** colonies are smooth, convex, opaque, easily emulsifiable, produce golden-yellow non-diffusible pigments made of β -carotene
- **Nutrient agar slope:** golden-yellow colonies of confluent growth [oil paint appearance]
- **Blood Agar:** narrow zone of β -hemolysis
- **MacConkey Agar:** small pink colonies
- **Selective media:** mannitol salt agar, salt milk agar, Ludlam's medium
- **Culture Smear Microscopy:** gram +ve cocci (hanging drop reveals non-motile cocci)
- **Biochemical Identification:**
 - **Catalase Test:** +ve
 - * **Coagulase Test:** +ve (differentiates S. aureus from CONS)
 - **Protein A detection:** by latex agglutination test (differentiates S. aureus from CONS)
 - * **Tube Coagulase Test:** positive test is indicated by clot formation
 - S. aureus is emulsified in plasma in a test tube & incubated at 37°C
 - detects free coagulase secreted by S. aureus
 - * **Slide Coagulase Test:** detects clumping factor (bound coagulase)
 - S. aureus is emulsified with drop of normal saline to form milky white suspension; then a loopful of plasma is added.
 - positive result indicated by formation of coarse clumps [negative $\Rightarrow$ remains as milk white suspension]
- **Identification by Automated Systems:** VITEK & MALDI-TOF
- **Antimicrobial Susceptibility Testing:** disc diffusion method

Treatment of S. aureus infections: use antibiotics should be used cautiously as S. aureus develops drug resistance rapidly

• Sensitive to penicillin	DOC: Penicillin G
• Methicillin sensitive S. aureus (MSSA)	DOC: anti-staphylococcal penicillins (Nafcillin, Cloxacillin)
• Methicillin resistant S. aureus (MRSA)	DOC: Vancomycin (15-20 mg/kg BD)
• Empirical therapy (when MRSA status is not known)	alternatives - linezolid, daptomycin Vancomycin +/- aminoglycoside

- **Drug Resistance in S. aureus:** S. aureus shows resistance to β -lactam antibiotics by production of β -lactamase/penicillinase enzyme which cleave the β -lactam rings
- this resistance is plasmid-coded (transferred b/w S. aureus strains by transduction)
- S. aureus (MRSA) also alters penicillin-binding protein (PBP)
 - mediated by a chromosomally coded gene called mecA gene
 - PBP is needed for cell wall synthesis
 - β -lactams bind & inhibit this protein
 - PBP is altered to PBP2a which has less affinity to β -lactam drugs $\therefore$ MRSA is resistant to β -lactam antibiotics

Detection of MRSA:

- Antimicrobial susceptibility test using ceftazidime disk or oxacillin
- PCR detecting mecA gene

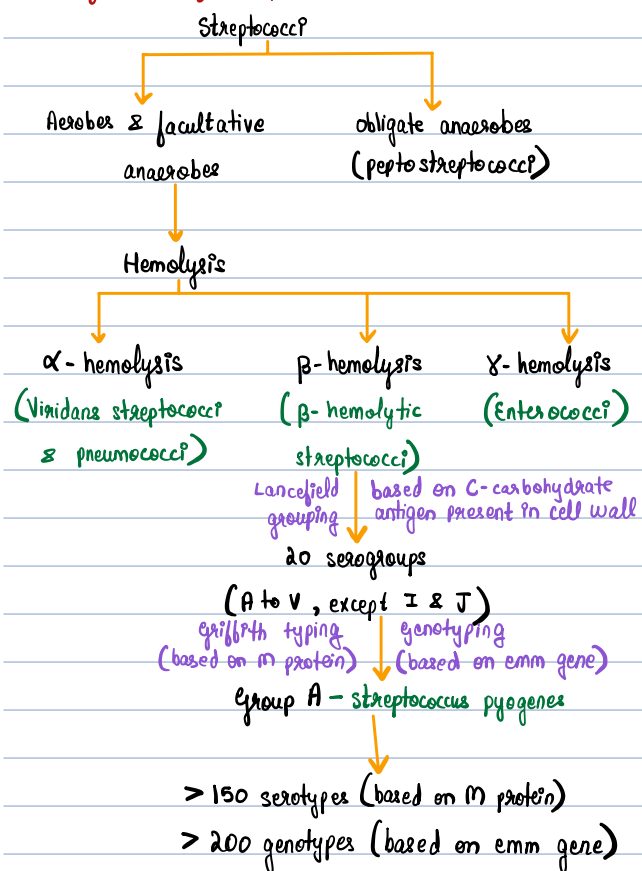
Types of MRSA:

- Hospital associated [HA-MRSA]: low virulence with multidrug resistance
- Community associated [CA-MRSA]: usually more virulent

β-hemolytic Streptococcal Infections

Streptococci: catalase -ve, gram +ve cocci, arranged in pairs or chains

Classification of Streptococci:



Streptococcus Pyogenes Infections:

→ S. pyogenes is the only species under Lancefield's group A streptococcus (GAS).

Virulence Factors: Cell wall antigens, toxins, enzymes

Cell Wall Antigens:

- **Teichoic acid:** helps in adhesion to host cells & proteins
- **C-carbohydrate antigens**
- **M proteins:** principle virulence factor
 - ↳ inhibits complement mediated opsonization by phagocytes
- **Fimbriae & F factor:** help in adhesion
- **M protein-associated protein (opacity factor):** associated with skin infections
- **Capsule:** present in some strains of GAS
 - ↳ made of hyaluronic acid
 - ↳ it is antiphagocytic & helps GAS to colonize the pharynx by binding to CD44

Toxins:

- **hemolysins:** streptolysin-O & streptolysin-S
 - ↳ cause RBC membrane lysis leading to complete β-hemolysis surrounding the colonies
- **Enzymes:**
 - **Streptokinase (fibrinolysin):** activates plasminogen to plasmin ∴ breaks down fibrin barrier around the infected site ⇒ facilitates spread of infection.
 - Therapeutic use: treatment of MI & other thromboembolic disorders
 - **Streptodornase (DNase):** breaks down DNA ∴ liquefies thick pus (containing large amount of DNA derived from nuclear acid of necrotic cells) & may be responsible for serious nature of streptococcal exudates (watery secretion).
 - Therapeutic use: used to liquefy thick exudates in empyema cases
 - **Hyaluronidase (spreading factor):** breaks down hyaluronic acid in tissues ∴ helps in spreading of infection along intercellular spaces.

Streptolysin [SL-O]	Streptolysin [SL-S]
• Oxygen labile, heat labile • Strongly antigenic • Anti-streptolysin O antibodies (ASO) are raised in most streptococcal infections; used as standard marker for retrospective diagnosis of streptococcal infections (except in glomerulonephritis & pyoderma where ASO titre is low)	• Oxygen stable, serum soluble • Not antigenic • Not useful for serological diagnosis of streptococcal infections

Clinical Manifestations: GAS produce both suppurative & non-suppurative manifestations

→ throat is the primary site of invasion by GAS (transmission through respiratory droplets)

Suppurative Infections:

① **Respiratory Infections:** S. pyogenes causes pharyngitis (sore throat)

→ Infection occurs through respiratory droplet

→ S. pyogenes rarely causes scarlet fever (pharyngitis & rashes), pneumonia or empyema.

② **Superficial Skin & Soft Tissue Infections:**

① **Impetigo (Pyoderma):**

→ Risk factors: young children, warmer months, tropical climates, poor hygiene, minor trauma

→ most common sites involved: face (nose & mouth) & legs

→ red papules → vesicles → pustular lesions

→ pustular lesions break down & coalesce to form thin papery honeycomb-like crusts

→ lesions are painless; no fever

② **Cellulitis:** involves skin & s.c. tissue

③ **Erysipelas:** indurated peau d'orange texture of involved skin (due to involvement of superficial lymphatics)

→ fevers & chills

→ superficial blebs or bullae

→ most common sites: face, lower extremities

③ **Deep Soft Tissue Infections:**

① **Necrotizing Fasciitis:** (Hemolytic streptococcal gangrene)

→ Source of infection:

- traumatized skin

- G.I.T breach (due to abdominal surgery)

→ GAS is the most common cause of necrotizing fasciitis

→ onset is acute & rapid

→ severe pain with minimal erythema at site of involvement

→ malaise, fever, chills, toxic appearance of skin

→ skin becomes dusky or develops mottled erythema & becomes anaesthetized with extreme necrosis of s.c. tissue (∴ GAS is also called flesh-eating bacteria)

② **Bacteraemia:** occurs secondary to necrotizing fasciitis

↳ leads to a variety of focal infections including endocarditis, meningitis, septic arthritis, peritonitis, osteomyelitis, visceral abscesses & toxic shock syndrome

③ **Toxic Shock Syndrome:** criteria: • isolation of S. pyogenes + • hypotension + • multiorgan (≥ 2) involvement

Non-Suppurative Complications:

→ Streptococcal antigens show molecular mimicry with human antigens

→ due to antigenic cross-reactivity, antibodies produced against previous streptococcal infections cross react with human tissue to produce lesions

- Acute rheumatic fever
- Post-streptococcal glomerulonephritis (PSGN)
- Guttate psoriasis
- Reactive arthritis
- Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal pyogenes (PANDAS) (⇒ - cross reactivity)

Streptococcal Antigen	Human Antigen
• Cell wall M protein (of serotypes M1, M5, M6, M19)	⇒ Myocardium (tropomyosin & myosin)
• Cell wall C carbohydrate	⇒ Cardiac valves
• Cytoplasmic membrane	⇒ Glomerular vascular intima
• Peptidoglycan	⇒ skin antigens
• Hyaluronic acid	⇒ synovial fluid

① **Acute Rheumatic Fever:** occurs in people previously infected with GAS sore throat caused due to autoimmune reaction.

② **PSGN:** typically occurs in children after streptococcal pyoderma

→ characterized by lodging of antigen-antibody complexes on glomerular basement membrane followed by complement activation

→ patients have elevated streptococcal anti-DNase B antibodies.

Laboratory Diagnosis:

→ Common specimens: pus swabs, exudates, blood (depends on the site of infection)

• **Direct Smear Microscopy:** pus cells with gram +ve cocci in chains

• **Culture:** (hanging drop reveals non-motile cocci)

→ S. pyogenes is fastidious (does not grow on MacConkey agar & basal media like nutrient agar or peptone water broth)

→ it grows only in media enriched with blood, serum or carbohydrate.

- **Blood Agar:** pinpoint colony with wide zone of β-hemolysis

- **Selective media:** Crystal violet blood agar.

• **Biochemical Tests:**

- **Catalase Test:** catalase -ve

- **Bacitracin Sensitivity Testing:** GAS is sensitive to bacitracin 0.04 U; most other β-hemolytic streptococci are resistant

Treatment: Penicillin is the DOC for pharyngeal & suppurative infections

→ in case of penicillin allergy ⇒ macrolides

Characters	S. pyogenes	S. agalactiae
Lancefield Grouping	Group A	Group B
Bacitracin sensitivity testing	Sensitive	Resistant
CAMP test	Negative	Positive
β-hemolytic colonies	pinpoint colonies	mucoid, slightly larger

CAMP test: Christie, Atkins & Munch-Peterson Test

Clostridial Infections:

- gram +ve bacilli with bulging spores
- *C. perfringens*: causes gas gangrene (an edematous myonecrosis like condition)
- *C. tetani*: causes tetanus (mediated by tetanus toxin which causes skeletal muscle spasm & autonomic nervous system disturbance)
- *C. botulinum*: produces botulinum toxin which causes flaccid paralysis of muscles
- *C. difficile*: causes pseudomembranous colonization of hospitalized patients with h/o prolonged intake of broad spectrum antibiotics.
- Spores in Clostridium: most Clostridia have a subterminal spore except -
 - *C. bifementans* ⇒ central & oval spores
 - *C. tetani* ⇒ spherical & terminal spore [Drum-stick appearance]
 - *C. tetium* ⇒ oval & terminal spores [tennis-racket appearance]
- In RCM broth: Clostridia grow well & produce turbidity in the medium
 - Proteolytic clostridia turn meat black & produce foul odour *C. tetani*, *C. botulinum* A, B, F
 - Saccharolytic Clostridia turn meat pink *C. perfringens*, *C. difficile*, *C. botulinum* C, D, E.

Clostridium Perfringens

- commensal in large intestine
- capsulated, gram positive, non-motile bacilli
- subterminal bulging spores

Virulence Factors:

- Four major toxins - α , β , ϵ , ι
- 8 minor toxins
- heat labile enterotoxin
- neuraminidase histamine, bursting factor (produces muscle lesions) & circulating factor (antiphagocytic).

Clinical Manifestations:

Clostridial Wound Infections:

- Simple wound contamination: wound surface contamination without invasion of underlying tissue
- Anaerobic cellulitis: involves fascial plane with minimal toxin release, without muscle invasion
- Anaerobic myositis (gas gangrene): muscle invasion occurs, gas in the muscle compartments with abundant toxin release.

Clostridial Enteric Infections:

- Food poisoning: caused by *C. perfringens* type A enterotoxin
 - source: contaminated meat
 - enterotoxin acts by forming pores in the intestinal mucosal membrane.
- Enteritis necroticans (gas gangrene of the bowel): characterized by ischemic necrosis of jejunum & gas in the tissue plane
 - caused by *C. perfringens* type C strains which produce β toxin.
- Necrotizing enterocolitis
- Gangrenous appendicitis
- Other Clostridial Infections:
 - Bacteremia
 - Panophthalmitis
 - Skin & soft tissue infections
 - Infection of endometrium leading to TSS
 - Meningitis & brain abscess

Gas Gangrene: rapidly spreading, edematous myonecrosis occurring in association with severely crushed wounds contaminated with pathogenic clostridia (particularly *C. perfringens*)
→ aka malignant edema/clostridial myonecrosis.

Etiological Agents: gas gangrene is always polymicrobial & caused by multiple Clostridia species

- Established agents: *C. perfringens*, *C. septicum*
- Probable agents: *C. histolyticum*, *C. tetium*

Pathogenesis: development of gas gangrene requires:

- Anaerobic environment: anoxic muscles start utilizing pyruvate anaerobically to produce lactic acid ⇒ crushing injuries of muscles such as RTA, foreign bodies, devitalized tissues leading to interruption in the blood supply & tissue ischaemia.
- Contamination of wound: with clostridial spores
- rarely, spontaneous non-traumatic gas gangrene occurs via hematogenous seeding of normal muscle with bowel clostridia.

Virulence Factors Mediating Gas Gangrene:

Toxins Produced by *C. perfringens*:

(i) α -toxin: principle virulence factor

- ↳ activates platelet adhesion molecule Gp11b/IIIa & neutrophil receptors CD41b/CD18 leading to formation of aggregates of platelets & neutrophils causing blood vessel occlusion
- suppresses myocardial contractility ⇒ ↓ cardiac output ⇒ profound hypotension

(ii) θ -toxin: causes marked vasodilation by activating mediators

Clinical Manifestations of Gas Gangrene:

- incubation period: ⇒ 10-48 hours for *C. perfringens*
⇒ 2-3 days for *C. septicum*
⇒ 5-6 days for *C. novyi*.
- various manifestations:
 - sudden onset of excruciating pain at affected site
 - rapid development of foul-smelling thin sero-sanguinous discharge
 - crepitus (gas bubbles)
 - brawny edema & induration
 - shock & organ failure develop later
 - mortality rate = 50%.

Laboratory Diagnosis:

- Specimen: necrotic tissues, muscle fragments, exudates from deeper parts of wounds
- Direct microscopy: absence of neutrophils in the infection tissues
 - thick, stubby, boxcar-shaped gram +ve bacilli without spore ⇒ suggestive of *C. perfringens*
 - chain bodies (boat or leaf-shaped pleomorphic irregularly stained bacilli with spores) ⇒ suggest *C. septicum*
- Identification: Target hemolysis (double zone hemolysis) on blood agar, *C. perfringens* produce an inner narrow zone of complete hemolysis (due to θ -toxin),

surrounded by a wider zone of incomplete hemolysis (due to α -toxin)

Nagler's Reaction: *C. perfringens* produces an opalescence surrounding the streak line on egg yolk agar (due to lecithinase activity of α -toxin)

Reverse CAMP test: *C. perfringens* is streaked over centre of blood agar plate & *S. agalactiae* is streaked perpendicular to it.
→ positive test: presence of enhanced zone of hemolysis (arrow-shaped) pointing towards *C. perfringens*.

Anthrax

- *Bacillus anthracis*: spore-forming, obligate aerobe
- humans acquire the infection through contact with an affected animal or by inhaling spores
- Historical importance**: first pathogenic bacterium to be seen under the microscope
- first bacterium to be isolated in pure culture by Robert Koch
- Anthrax vaccine was the first live attenuated bacterial vaccine prepared.

Virulence Factors & Pathogenesis:

- **Anthrax Toxin**: tripartite toxin consisting of 3 fragments -
 - **Edema factor**: acts as adenyl cyclase & causes edema
 - **Protective factor**: binding fragment that binds to the host cell receptors & facilitates entry of other fragments into the host cell.
 - **Lethal factor**: causes cell death
- The 3 factors together are active & produce local edema & generalized shock
- Toxin synthesis is controlled by a plasmid (pX01)
- Loss of plasmid makes the strain avirulent
- **Anthrax Capsule**: polypeptide capsule made up of polyglutamate
 - capsule is plasmid (pX02) coded
 - it inhibits complement mediated phagocytosis.

Clinical manifestations:

- **Transmission**: cutaneous mode, inhalation of spores, ingestion of carcasses of animals dying of anthrax containing spores.
- Clinical types - Cutaneous anthrax
 - Pulmonary anthrax.
 - Intestinal anthrax
- *B. anthracis* is the most common agent of bioterrorism

Laboratory Diagnosis:

- **Specimen**: pus, sputum, blood, CSF
- **Direct Demonstration**: gram +ve, large rectangular bacilli
 - **McFadyen's reaction**: shows amorphous purple capsule surrounding blue bacilli on staining with Guze's polychrome methylene blue stain for 30 seconds
 - **Ascoli's thermoprecipitation test**: ring precipitation test performed when specimens are received in putrid form.
- **Culture**: aerobic, non-fastidious
 - grows in ordinary media & has a wide temp. range (12-45°C)
 - **Nutrient Agar**: grayish white colonies with a frosted glass appearance (Medusa head appearance when viewed under low power)
 - **Blood agar**: dry wrinkled, non-hemolytic colonies
 - **Gelatin stab agar**: inverted fir tree appearance
 - **Selective media**: - solid medium with penicillin
 - **PLET medium**: contains polymyxin, lysozyme, EDTA, thallous acetate added in heart infusion agar.

- **Culture Smear**: gram +ve bacilli with non-bulging spores (bamboo stick appearance)

→ spores can be demonstrated using hot malachite green (Ashby's method) or modified acid-fast staining using 0.25% H_2SO_4 .

- **Molecular Diagnosis**: PCR with specific primers.

Treatment:

- **Cutaneous anthrax**: Amoxicillin, ciprofloxacin or doxycycline for 7-10 days

Classification of Non-Sporing Anaerobes:

Gram +ve cocci

- *peptostreptococcus*
- *peptococcus*

Gram +ve bacilli

- *Bifidobacterium*
- *Eubacterium*
- *Propionibacterium*
- *Lactobacillus*
- *Actinomyces*
- *Mobiluncus*

Gram -ve cocci

- *veillonella*

Gram -ve bacilli

- *Bacteroides*
- *Prevotella*
- *Porphyromonas*
- *Fusobacterium*
- *Leptotrichia*

Spirochetes

- *treponema*
- *borelia*

Varicella Zoster Virus Infection (VZV)

→ causes vesicular infections on skin & mucous membrane

→ Chickenpox is characterized by bilateral vesicular rashes

→ Zoster or Shingles: occurs following reactivation of VZV present in trigeminal ganglia

- vesicular rashes are unilateral & segmental

Pathogenesis: VZV enters through upper respiratory mucosa or conjunctiva by aerosol & contact transmission → VZV spills over to blood

→ VZV is carried through infected mononuclear cells to:

- skin (produces rashes)
- respiratory tract (shed in respiratory secretion)
- Neurons (undergoes latency)

→ incubation period = 10-21 days

Clinical Manifestations: characterized by rashes

→ rashes are vesicular & centripetal in distribution

→ bilateral & diffuse in distribution

→ rashes appear in multiple crops in varying stages — maculopapules, vesicles, pustules & scabs

→ fever appears with each crop of rashes

→ it is a disease of childhood

Complications: more common in adults & immunocompromised individuals & they are:

- Secondary bacterial infections of skin
- CNS involvement (in children) (cerebral ataxia, encephalitis & aseptic meningitis)
- Varicella pneumonia (in adults), particularly severe in pregnant women
- Reye's Syndrome: can occur secondary to VZV infection; characterized by fatty degeneration of liver following salicylate (aspirin) intake
- myocarditis, nephritis, corneal lesions
- Chickenpox in pregnancy: can affect both mother & fetus

— mothers are at high-risk of developing varicella pneumonia

— fetus is at higher risk of developing congenital varicella syndrome characterized by cicatricial lesions & limb hypoplasia

Epidemiology: chickenpox is highly contagious

→ primarily a disease of temperate regions

→ common in children (1-14 years of age)

→ one attack gives lifelong immunity

→ humans are the only known reservoir

Zoster / Shingles / zema: usually occurs due to reactivation VZV in old age (>60 yrs)

→ usually starts with severe pain in the area of skin or mucosa supplied by sensory nerves & ganglia of the nerve involved

→ rashes are unilateral & segmental

→ most common nerve involved = ophthalmic branch of trigeminal nerve which affects the head, neck & trunk

Complications of Zoster:

→ post-herpetic neuralgia (pain at the local site lasting for months)

→ zoster ophthalmicus (unilateral painful crops of skin rashes surrounding the eye)

→ Ramsay Hunt Syndrome: develops when geniculate ganglion of facial nerve is involved; characterized by a triad of ipsilateral facial paralysis, ear pain & vesicles on the face, tympanic membrane & external auditory meatus

→ Visceral disease like pneumonia

Laboratory Diagnosis:

→ Specimens: vesicular lesions, scabs, maculopapular lesions

→ Giemsa staining of scrapings from the ulcer base (Tzanck smear) shows formation of multinucleated giant cells

→ specific antigen detection by direct immunofluorescence staining

→ specific IgM & IgG antibody detection by ELISA

→ PCR detecting VZV-specific genes

Treatment: Acyclovir, Famciclovir, Valacyclovir

Vaccine: live attenuated vaccine using OKa strain

→ given to children after 1 year of age — first dose at 12-15 months & second dose at 4-6 years

- VZIG (Varicella Zoster Immunoglobulin): useful for post-exposure prophylaxis, given within 96 hours of exposure
- recommended to adults, immunocompromised, pregnancy, neonates born to mothers suffering from chickenpox (if the onset of chickenpox in mother is < 5 days before delivery till 48 hours after delivery)

Parvovirus Infections: Cause a common childhood exanthema called erythema infectiosum (Fifth disease)

→ smallest viruses, non-enveloped with icosahedral symmetry, possess ssDNA

→ Parvovirus B19 is most pathogenic to man

Pathogenesis:

→ transmission occurs by respiratory route followed by blood transfusion & transplacental route

→ Parvovirus B19 has a special tropism for erythroid progenitor cells in adult bone marrow & fetal liver as it binds to blood group P antigen as receptors present on RBC surface

→ this causes destruction of RBCs & inhibition of erythropoiesis.

Clinical Manifestations:

→ Erythema infectiosum (Fifth Disease): rashes on the face with characteristic slapped cheek appearance in children; adult women present with symmetrical polyarthralgia involving hand joints & knee

→ Transient aplastic crisis: can occur in infected patients with underlying hemolytic anemia

→ Pure red cell aplasia: can occur in those with underlying immunosuppression.

→ Non-immune hydrops fetalis: in fetus due to transplacental transmission

→ Papular-purpuric gloves & Socks Syndrome: presents as rapidly progressive, painful, pruritic & symmetric swelling & erythema of distal hands & feet

Laboratory Diagnosis:

• Molecular methods: PCR detecting viral DNA

• Antibody detection: ELISA for detecting antibodies against VP1 & VP2 antigens

• Antigen detection: immunohistochemistry

Treatment: no available antiviral drug; symptomatic treatment is given.

Human Papillomavirus (HPV) Infection:

→ DNA virus of Papillomaviridae family which has selective tropism for epithelium of skin & mucous membranes.

Clinical Manifestations:

Benign warts: small, hard, rough growth on the skin.

→ It is of the following types:

- Common skin warts (verruca vulgaris) & flat warts (verruca plana): seen in children
- Plantar warts (verruca plantaris): seen in adolescents
- Anogenital warts (condyloma acuminatum): sexually transmitted infection seen in adults

Poxvirus Infection: largest viruses (can be seen under light microscope)

→ complex symmetry (neither icosahedral or helical)

→ brick-shaped / ellipsoid

→ envelope: made of 2 lipoprotein membranes with ridges arising from the outer membrane

→ core / nucleocapsid is biconcave dumbbell-shaped

→ single linear dsDNA

• Varicella: smallpox causative agent

• Vaccinia: used as a vaccine for smallpox

• Molluscum contagiosum virus: warty lesions called molluscum contagiosum

Smallpox: 1st infectious disease to be eradicated from the world

→ characterized by highly contagious severe exanthema (rashes)

→ caused by Variola major & variola minor

Clinical Manifestations: portal of entry of virus was via mucous membranes of upper respiratory tract (aerosol transmission)

→ incubation period: 7-17 days

→ smallpox rashes are unique in appearance

• rashes are deep-seated & all rashes in an area appear in one stage

→ centrifugal distribution of rashes

→ fever subsides with appearance of rashes.

Laboratory Diagnosis:

• Direct detection in scrapings from rashes:

- intracytoplasmic inclusion bodies (Paschen bodies)
- electron microscopy

• Egg inoculation: characteristic pock formation is seen on chorioallantoic membrane (CAM) of a chick embryo

Treatment: vaccinia immunoglobulins & antiviral drugs (methisazone, cidofovir, tecovirimat)

Vaccination:

- Live Vaccinia vaccine: single dose given at 1-2 years of age
- Camxox Vaccine: discovered by Edward Jenner
- Variolation: first attempt at providing artificial immunity against smallpox.

Smallpox	Chickenpox
Incubation period: 7-17 days	Incubation period: 10-21 days
Site of rash: palm, sole, extensor surface	Site of rash: axilla & flexor surface
Rash: deep-seated & appear in single stage, centrifugal distribution	Rash: superficial, pleomorphic, dew drop rashes, centripetal distribution
Fever subsides with appearance of rash	Fever rises with each crop of rash.

Measles: acute, highly contagious disease of childhood

→ characterized by fever & respiratory symptoms followed by typical maculopapular rash

→ caused by Measles virus (RNA virus) ⇒ Paramyxoviridae family

Pathogenesis:

→ transmission via respiratory route either by droplet inhalation or small-particle aerosols.

→ virus multiplies locally in respiratory tract → spread to regional lymph nodes → enters into bloodstream through infected monocytes → multiplies in reticuloendothelial system → spills over to blood → dissemination to various sites

→ virus is predominantly seeded in epithelial surfaces of the body

Clinical Manifestations: incubation period: 10 days - 3 weeks

→ 3 stages of disease

• **Prodromal Stage:** lasts for 4 days (10th-14th day)
→ characterized by fever on day 1 (10th day of infection) & Koplik's spots 2 days after fever (12th day)

→ **Koplik's spots:**

- white to bluish spot surrounded by erythema
- appears first on buccal mucosa near lower and molars
- rapidly spreads to rest of buccal mucosa

→ cough, coryza, nasal discharge, redness of eye, diarrhoea, vomiting

• **Exanthematous Stage:** maculopapular dusky red rashes 4 days after fever (14th day)

→ rashes typically appear behind the ear → spread to face, arm, trunk, legs → fade in the same order 4 days after onset

• **Post-measles Stage:** weight loss & weakness

Complications: children < 5 years, adults > 20 years, pregnant women, immunocompromised hosts.

• **Secondary Bacterial Infections:** otitis media, bronchopneumonia, worsening of underlying TB.

• **Complications due to Measles Virus Itself:**

→ Giant cell pneumonia (Meckel's pneumonia) in immunocompromised hosts, acute laryngo-tracheobronchitis (croup), diarrhoea leading to malnutrition

• **Central Nervous System Complications:**

→ subacute sclerosing panencephalitis (SSPE), post-measles encephalomyelitis.

Laboratory Diagnosis:

→ **Specimens:** nasopharyngeal swabs, conjunctival swabs, blood, respiratory secretions, urine

• **Antigen Detection:** by direct immunofluorescence test

• **Virus Isolation:** using monkey or human kidney cell lines

→ cytopathic effect may be observed after 7-10 days of inoculation into cell line characterized by multinucleated giant cells (Warthin-Finkeldey cells) containing both intranuclear & intracytoplasmic inclusion bodies

• **Antibody Detection:** detection of measles-specific IgM by ELISA

• **Reverse transcriptase PCR:** targeting measles specific RNA such as N gene

Treatment: no specific antiviral therapy

→ symptomatic treatment & supportive measures

Measles Vaccine: live attenuated vaccine

→ strains used: - Schwartz strain - Moraten strain - Edmonston-Zagreb strain.

→ vaccine is prepared in chick embryo cell line.

→ vaccine is available in lyophilized form & has to be reconstituted with distilled water & should be used within 4 hours.

→ vaccine is stored at -20°C (∵ it is thermolabile)

→ One dose (0.5 mL)

→ Measles vaccine available in combined form:

- MR (measles, rubella)
- MMR (measles, mumps, rubella)
- MMR-V (MMR + varicella)

→ MR vaccine is given at 9-12 completed months along with vitamin A supplements & 2nd dose at 16-24 months.

→ cases are the only source of measles infection

→ humans are the only reservoir of infection

→ virus is shed in the nasal, throat & respiratory tract secretions

Rubella: Rubella virus produces a childhood exanthema similar to measles ∴ aka German measles

→ highly teratogenic & can cause congenital rubella syndrome.

→ family Togaviridae, genus Rubivirus

→ ssRNA surrounded by a capsid (C) protein & envelope.

→ Rubella may present in 2 clinical forms:

- postnatal infection
- congenital infection

POST-NATAL RUBELLA: may occur during neonatal age, childhood & adult life

→ spreads from person-to-person by respiratory droplets via upper respiratory mucosa

→ Rubella virus replicates locally in nasopharynx → spreads to lymph nodes → viremia develops after 7-9 days & lasts till 14th day by which time antibody & rash appear.

Clinical Manifestations: incubation period: 12-23 days

→ Rashes ⇒ generalized & maculopapular, start on the face, disappear in 3 days

→ occipital & post-auricular lymphadenopathy

→ Forchheimer spots: pin-head sized petechiae, develop on soft palate & uvula

Complications: arthralgia, arthritis, thrombocytopenia, encephalitis

Laboratory Diagnosis:

- **Isolation of virus:** nasopharyngeal/throat swabs ⇒ monkey or rabbit origin cell lines

- **Serology:** antibody detection by ELISA

- **Molecular method:** RT-PCR for detecting rubella-specific RNA

CONGENITAL RUBELLA SYNDROME:

→ affects ear (deafness), eye (cataract), heart (patent ductus arteriosus)

→ severity is maximum in 1st trimester.

→ cases are the only sources of infection (no known carrier state)

→ one infection provides life long immunity

→ transmission: airborne droplet, transplacental, contact, sexual modes

→ no specific treatment available

Rubella Vaccine: RA 27/3 ⇒ live attenuated vaccine

prepared from human diploid fibroblast cell line

→ single dose (0.5 mL) administered subcutaneously

→ given along with measles (MR vaccine) at 9-12 months & second dose at 16-24 months

→ vaccine is contraindicated in pregnancy

Hand-Foot-&-Mouth Disease (HFMD):

→ usually affects children

→ characterized by ulcerations on oral & pharyngeal mucosa & vesicular rashes on the palm & soles which heal without crusting

→ fever & sore-throat (flu-like symptoms)

→ caused by Coxsackieviruses

→ Coxsackievirus B16 is most common cause.

→ transmission: via infected person's nose & throat secretions through contact & droplets.

Herpes Virus: family herpesviridae

- Icosahedral symmetry
- linear dsDNA surrounded by a capsid
- nucleocapsid is surrounded by a lipid envelope into which glycoprotein spikes are embedded
- between the capsule & envelope, there is an amorphous asymmetric structure called tegument.

Pathogenesis:

- Virus replicates at local site of infection → virus invades the local nerve endings → virus is transported by retrograde axonal flow to dorsal root ganglia → replicates further here → undergoes latency → reactivation of latent virus can occur following various stimuli (fever, axonal injury, physical or emotional stress) → via axonal spread, virus goes back to peripheral site & replicates further → secondary lesions

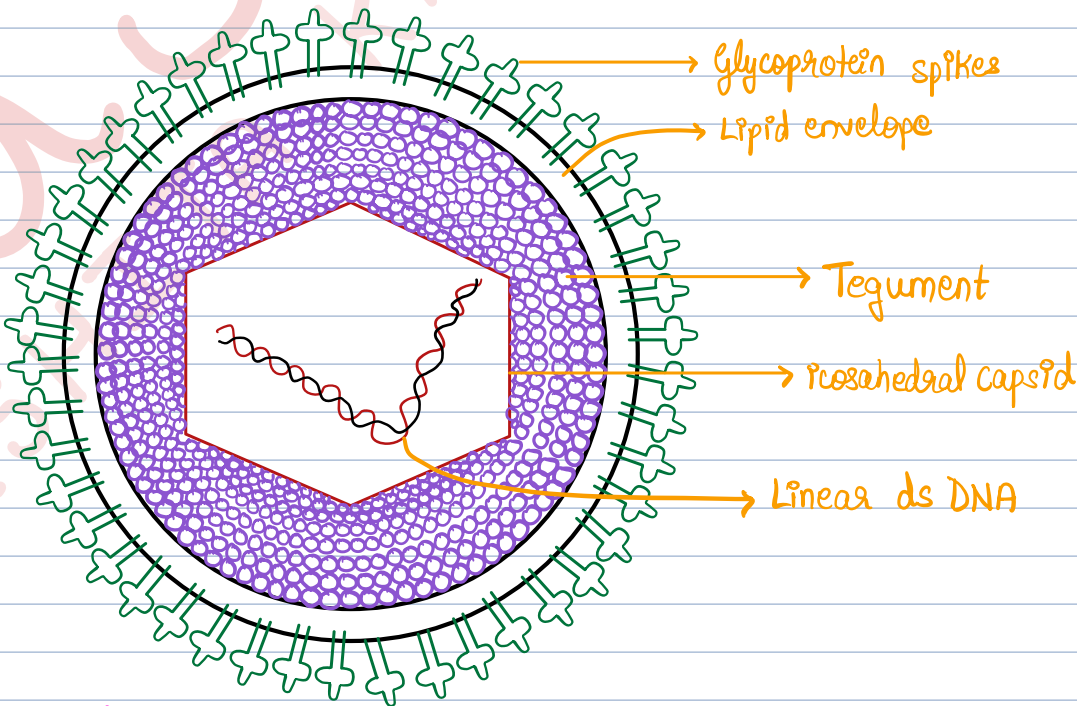
Laboratory Diagnosis:

- Cytopathology (Tzanck preparation): (by Wright's or Giemsa stain) detects inclusion bodies (Lipschultz body) & formation of multinucleated giant cells
- Virus Isolation by:
 - Conventien cell lines — used to demonstrate diffuse rounding & ballooning of cell lines
 - shell vial culture
- Viral antigen detection: by direct IF
- HSV DNA detection: by PCR
- Antibody detection: by ELISA

Treatment: DOC: Acyclovir (it acts by inhibiting viral DNA polymerase)
→ in case of Acyclovir resistance, DOC = Foscarnet.

subfamily (- herpesvirinae)	Duration of replication & cytopathology	Site of latency	Genus	Species	
				official Name	Common Name
Alpha	Short (12-18 hours) Cytolytic	Neurons	Simplexvirus	Human herpesvirus 1	Herpes simplex virus 1
				Human herpesvirus 2	Herpes simplex virus 2
			Varicellovirus	Human herpesvirus 3	Varicella - zoster virus
Beta	Long (> 24 hours) Cytomegalic Long (> 24 hours) Lymphoproliferative	Glands, kidneys Lymphoid tissues (T cells)	Cytomegalo- virus	Human herpesvirus 5	Cytomegalovirus
			Roseolovirus	Human herpesvirus 6	Human herpesvirus 6
				Human herpesvirus 7	Human herpesvirus 7
Gamma	Variable Lymphoproliferative	Lymphoid tissues (B cells)	Lymphocrypto- virus	Human herpesvirus 4	Epstein - Barr Virus
			Rhadinovirus	Human herpesvirus 8	Kaposi's sarcoma - associated herpesvirus

Properties	HSV 1	HSV 2
Common modes of transmission	direct contact with mucosa or abraded skin	Sexual mode or vertical mode
Latency in	Trigeminal ganglia	Sacral ganglia
Age affected	Young children	Young adults
Common manifestations	• orol-facial mucosal lesions • Encephalitis & meningitis • Ocular lesions • Skin lesions above waist	• Genital lesions • Skin lesions below waist • Neonatal herpes
Neurovirulence	Less	More
Drug Resistance	Less	More
Antigenic homology	HSV 1 & 2 show > 80% antigenic homology	
DNA homology	HSV 1 & 2 show > 50% homology in the genomic sequence	



HERPES SIMPLEX VIRUS