1. (a) Mention the contributions of the following Scientists (Any four)
    - (i) Elie Metchnikoff
        - Known for his pioneering research in immunology.
        - Discovered phagocytes and elucidated the process of phagocytosis, which is the engulfment of microorganisms by immune cells.
        - His work laid the foundation for understanding innate immunity.
    - (ii) Alexander Fleming
        - Discovered penicillin, the first widely effective antibiotic.
        - Observed the inhibitory effect of *Penicillium notatum* mold on bacterial growth.
        - Revolutionized the treatment of bacterial infections.
    - (iii) Charles Chamberland
        - Developed the Chamberland filter, a porcelain filter that could remove bacteria from liquids.
        - This invention was crucial for sterilizing liquids and for studying viruses, as it allowed for the separation of bacteria from smaller infectious agents.
    - (iv) Edward Jenner
        - Pioneered the concept of vaccination.
        - Developed the world's first vaccine, for smallpox, using cowpox material.
        - His work laid the foundation for modern vaccinology and eradicated smallpox.
    - (v) Robert Hooke

- Coined the term "cell" after observing cork tissue under a microscope.
- Published "Micrographia," a book containing detailed drawings of his microscopic observations.
- Contributed significantly to the early understanding of microscopic life.

2. (b) Mention the causative organisms of the following diseases (Any three)
    - (i) Pertussis
        - *Bordetella pertussis*
    - (ii) Leprosy
        - *Mycobacterium leprae*
    - (iii) Gonorrhea
        - *Neisseria gonorrhoeae*
    - (iv) Tetanus
        - *Clostridium tetani*
3. (c) Give one word for the following (Any five)
    - (i) The bacteria which are able to grow at 10°C to 0°C but which optimally grow at 30°C.
        - Psychrotrophs
    - (ii) Endotoxin released by a gram negative organism.
        - Lipopolysaccharide (LPS)
    - (iii) Technique employed to create an oxygen free environment for bacteria that cannot survive or thrive when oxygen is present.

- Anaerobic jar/Anaerobic chamber

- (iv) A culture medium in which the exact chemical composition is known.
  - Chemically defined medium (or Synthetic medium)
- (v) The transfer of genetic material from one cell to another involving cell-to-cell contact.
  - Conjugation
- (vi) Lowest concentration of a toxic substance in an environmental medium that kills individual organisms or test species under a defined set of conditions
  - Lethal concentration (LC)
- (vii) Study of genomes recovered from environmental samples without isolating members of microbial community and growing them in pure cultures.
  - Metagenomics

4. (d) Justify the statement: (Any three)
   - (i) MacConkey agar is both selective and differential media which is used for the growth of microorganisms.
     - **Selective:** MacConkey agar contains bile salts and crystal violet, which inhibit the growth of gram-positive bacteria, thus selecting for gram-negative bacteria.
     - **Differential:** It contains lactose and a pH indicator (neutral red). Lactose-fermenting bacteria produce acid, lowering the pH and turning the colonies pink or red, while non-lactose fermenters remain colorless or pale. This allows differentiation between different types of gram-negative bacteria.

- (ii) Concentration of 75% ethanol is more effective than 100% ethanol as a bactericidal agent.
    - 75% ethanol is more effective because water is essential for the denaturation of proteins and the disruption of cell membranes, which are the primary mechanisms of ethanol's bactericidal action.
    - 100% ethanol rapidly coagulates proteins on the outer surface of the cell, forming a protective layer that prevents deeper penetration of the alcohol, thus reducing its effectiveness. The presence of water in 75% ethanol allows for better penetration and more thorough denaturation of cellular components.
- (iii) It is important that the air initially present in the autoclave chamber is forced out.
    - Air in the autoclave chamber acts as an insulator and prevents the steam from reaching the desired sterilization temperature effectively.
    - If air is not completely purged, pockets of cooler air can remain, leading to incomplete sterilization of the load. Steam must fully penetrate all items for effective heat transfer and microbial killing.
- (iv) Carriers are important reservoirs in transmission of infection.
    - Carriers are individuals who harbor a pathogenic microorganism without showing overt signs or symptoms of the disease.
    - Despite being asymptomatic, they can shed the pathogen and transmit it to susceptible individuals, serving as a continuous source of infection within a population. This makes them critical in maintaining the chain of infection.

5. (a) Differentiate between the following (Any four):
    - (i) Hemagglutination and Plaque assay
        - **Hemagglutination:**
            - Measures the ability of certain viruses (e.g., influenza) to agglutinate red blood cells.
            - Indicates the presence of viral particles that can bind to RBCs, but does not distinguish between infectious and non-infectious virions.
            - Provides a quantitative measure of viral concentration, but not infectivity.
        - **Plaque Assay:**
            - Measures the number of infectious viral particles in a sample.
            - Based on the ability of a single infectious virus to infect a cell, replicate, and spread to surrounding cells, creating a visible clear zone (plaque) in a monolayer of host cells.
            - Provides a quantitative measure of infectivity, usually expressed as plaque-forming units (PFU).
    - (ii) Viroids and Prions
        - **Viroids:**
            - Small, circular, single-stranded RNA molecules.
            - Do not encode proteins.
            - Cause diseases primarily in plants.
            - Replicate autonomously within host cells.

- **Prions:**
  - Infectious proteins.
  - Do not contain nucleic acids (DNA or RNA).
  - Cause neurodegenerative diseases in animals and humans (e.g., Creutzfeldt-Jakob disease, mad cow disease).
  - Replicate by inducing misfolding of normal host proteins.

- (iii) Lethal Dose and Infectious Dose
  - **Lethal Dose (LD):**
    - Refers to the amount of a substance (e.g., toxin, chemical) or microorganism required to cause death in a specified percentage of the tested population.
    - Often expressed as LD50, meaning the dose that is lethal to 50% of the population.
    - Measures toxicity or virulence in terms of mortality.
  - **Infectious Dose (ID):**
    - Refers to the number of microorganisms required to cause infection in a specified percentage of the tested population.
    - Often expressed as ID50, meaning the dose that causes infection in 50% of the population.
    - Measures infectivity.

- (iv) Lysis and Lysogeny
  - **Lysis (Lytic Cycle):**

- A viral replication cycle where the bacteriophage replicates within the host cell and then lyses (breaks open) the host cell to release new virions.
- Results in the destruction of the host cell.
- Is characteristic of virulent phages.

▪ **Lysogeny (Lysogenic Cycle):**

- A viral replication cycle where the bacteriophage DNA integrates into the host bacterial chromosome, becoming a prophage.
- The prophage is replicated along with the host chromosome without causing lysis.
- The host cell survives and continues to grow.
- Is characteristic of temperate phages.

○ (v) Typhoid and Cholera

▪ **Typhoid:**

- **Causative Agent:** *Salmonella Typhi*.
- **Mode of Transmission:** Fecal-oral route, typically through contaminated food or water.
- **Symptoms:** High fever, headache, malaise, rose spots on the trunk, constipation or diarrhea, and potentially intestinal perforations.
- **Systemic Infection:** Affects multiple organs and can be severe.

▪ **Cholera:**

- **Causative Agent:** *Vibrio cholerae*.

- **Mode of Transmission:** Fecal-oral route, primarily through contaminated water.
- **Symptoms:** Profuse watery diarrhea ("rice-water stools"), vomiting, rapid dehydration, muscle cramps.
- **Localized Infection:** Primarily affects the small intestine, leading to severe fluid loss.

6. (b) Answer any one of the following:
    - (i) Discuss the factors contributing to the characteristic sigmoidal growth curve observed when bacteria grow in a batch culture.
        - The sigmoidal (S-shaped) growth curve in a batch culture typically consists of four distinct phases:

- **Lag Phase:**
    - No significant increase in cell number.
    - Bacteria are adapting to the new environment, synthesizing necessary enzymes and molecules.
    - The length depends on the previous growth conditions and the new medium.
- **Exponential (Log) Phase:**
    - Cells divide at a constant, maximal rate.
    - Cell number increases exponentially.
    - Growth is limited only by the intrinsic reproductive capacity of the bacteria and the availability of nutrients.

- This is the most uniform and metabolically active phase.

- **Stationary Phase:**
  - The rate of cell division equals the rate of cell death.
  - Net growth ceases, and the total viable cell count remains relatively constant.
  - Caused by depletion of essential nutrients, accumulation of toxic waste products, or changes in environmental conditions (e.g., pH).

- **Death Phase (Decline Phase):**
  - The rate of cell death exceeds the rate of cell division.
  - The number of viable cells decreases exponentially.
  - Caused by prolonged nutrient depletion and accumulation of toxic waste products, leading to irreversible cell damage.

- (ii) Discuss different types of flagellar movement in bacteria.
  - Bacterial flagella are responsible for motility and can exhibit different types of movement, primarily enabling "runs" and "tumbles." The movement is powered by a proton motive force.
  - **Runs:**
    - Occur when the flagella rotate counterclockwise (CCW).

- This rotation causes the individual flagellar filaments to form a unified bundle that propels the bacterium in a relatively straight line forward.
- Bacteria move smoothly in one direction during a run.

- **Tumbles:**
  - Occur when the flagella reverse their rotation to clockwise (CW).
  - This causes the flagellar bundle to fall apart, and the individual flagella move independently, leading to a random, erratic, and disorganized reorientation of the bacterium.
  - Tumbling allows the bacterium to change its direction of movement.

- **Chemotaxis:**
  - Bacteria use a combination of runs and tumbles to navigate towards attractants (e.g., nutrients) and away from repellents (e.g., toxins).
  - In the presence of an attractant, runs become longer and tumbles become less frequent, allowing the bacterium to move more directly towards the stimulus.
  - In the absence of an attractant or presence of a repellent, tumbles become more frequent, causing the bacterium to randomly reorient and search for a more favorable environment.

7. (a) What is the generation time of a bacterial population that increases from 10,000 cells to 10,000,000 cells in four hours of growth?

- Given:
  - Initial number of cells (N_0) = 10,000
  - Final number of cells (N_t) = 10,000,000
  - Time (t) = 4 hours = 240 minutes
- The formula for exponential growth is $N_t = N_0 \times 2^n$, where n is the number of generations.
- Rearranging the formula to find n:
  - $N_t/N_0 = 2^n$
  - $10{,}000{,}000/10{,}000 = 2^n$
  - $1000 = 2^n$
- To find n, take the logarithm base 2 of both sides:
  - $log_2(1000) = n$
  - Using $log_2(X) = ln(X)/ln(2)$:
  - $n = ln(1000)/ln(2) = 6.907/0.693 = 9.967$ (approximately 10 generations)
- Generation time (g) = total time / number of generations
  - $g = t/n$
  - $g = 240$ minutes$/9.967 \approx 24.08$ minutes
- The generation time of the bacterial population is approximately **24 minutes**.

8. (b) Discuss what are nosocomial infections. Give appropriate examples.
   - Nosocomial infections, also known as healthcare-associated infections (HAIs), are infections acquired by patients during the course of receiving medical care in a healthcare facility (e.g.,

hospitals, nursing homes, outpatient clinics) that were not present or incubating at the time of admission.

- These infections can affect any part of the body and can range from minor to life-threatening.
- **Common factors contributing to nosocomial infections:**
  - Compromised patient immunity due to underlying illness or medical procedures.
  - Invasive medical procedures (e.g., surgery, catheterization, ventilation).
  - Presence of antibiotic-resistant microorganisms in healthcare settings.
  - Breakdown in infection control practices (e.g., inadequate hand hygiene, improper sterilization of equipment).
- **Examples of nosocomial infections:**
  - **Urinary Tract Infections (UTIs):** Often associated with urinary catheterization, caused by bacteria like *Escherichia coli* or *Pseudomonas aeruginosa*.
  - **Surgical Site Infections (SSIs):** Infections occurring at the site of a surgical incision, frequently caused by *Staphylococcus aureus* or *Enterococcus* species.
  - **Pneumonia:** Hospital-acquired pneumonia (HAP), often ventilator-associated pneumonia (VAP), caused by various bacteria such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, or *Staphylococcus aureus*.
  - **Clostridioides difficile Infection (CDI):** An intestinal infection caused by *Clostridioides difficile*, often linked to antibiotic use that disrupts the normal gut flora.

- **Central Line-Associated Bloodstream Infections (CLABSIs):** Infections related to the presence of central venous catheters, often caused by *Staphylococcus epidermidis* or *Staphylococcus aureus*.

9. (c) Discuss how endospores are formed?

- Endospores are highly resistant, dormant structures formed by certain gram-positive bacteria (e.g., *Bacillus* and *Clostridium* species) in response to unfavorable environmental conditions, such as nutrient depletion or extreme temperatures. The process of endospore formation is called sporulation and involves several stages:
- **Steps of Endospore Formation (Sporulation):**
  - **Stage 0 (Vegetative Cell):** The cell is in its normal growing state.
  - **Stage I (Axial Filament Formation):** The bacterial chromosome replicates, and the two chromosomes move to opposite ends of the cell. An axial filament of nuclear material forms.
  - **Stage II (Septum Formation):** An asymmetric septum (membrane) forms near one end of the cell, dividing it into two unequal compartments: a larger mother cell and a smaller forespore (prespore). Each compartment receives a copy of the chromosome.
  - **Stage III (Engulfment):** The mother cell membrane engulfs the forespore, enclosing it within a second membrane. The forespore is now surrounded by two membranes.
  - **Stage IV (Cortex Formation):** Peptidoglycan is synthesized and laid down in the space between the two

membranes of the forespore, forming the cortex. This cortex is crucial for heat resistance.

- **Stage V (Coat Synthesis):** Protein layers are deposited on the outer surface of the forespore, forming the spore coat. This coat provides significant protection against chemicals, enzymes, and desiccation.
- **Stage VI (Maturation and Dipicolinic Acid Accumulation):** The spore fully matures. Dipicolinic acid (DPA) complexed with calcium ions accumulates in the core of the spore. This complex dehydrates the spore and stabilizes DNA, contributing significantly to heat resistance.
- **Stage VII (Lysis of Mother Cell):** The mother cell lyses, releasing the mature, free endospore into the environment.

- Endospores can remain viable for long periods and germinate back into vegetative cells when favorable conditions return.

10. (a) Discuss the differences between gram-negative and gram-positive cell wall?

- **Gram-Positive Cell Wall:**
  - **Peptidoglycan Layer:** Very thick (20-80 nm), consisting of multiple layers of peptidoglycan. It constitutes 60-90% of the cell wall.
  - **Teichoic Acids and Lipoteichoic Acids:** Present and extend through the peptidoglycan layer. Teichoic acids are polymers of glycerol or ribitol phosphate, while lipoteichoic acids are similar but anchored in the cytoplasmic membrane. They contribute to the cell's negative charge, regulate enzyme activity, and play a role in adhesion.

- **Outer Membrane:** Absent.
- **Periplasmic Space:** Absent or very narrow.
- **Lipopolysaccharide (LPS):** Absent.
- **Sensitivity to Penicillin/Lysozyme:** Generally more susceptible due to the accessible thick peptidoglycan layer.
- **Examples:** *Staphylococcus*, *Streptococcus*, *Bacillus*, *Clostridium*.

- **Gram-Negative Cell Wall:**
  - **Peptidoglycan Layer:** Relatively thin (2-7 nm), consisting of only one or a few layers of peptidoglycan. It constitutes 5-20% of the cell wall.
  - **Teichoic Acids and Lipoteichoic Acids:** Absent.
  - **Outer Membrane:** Present and external to the peptidoglycan layer. This outer membrane is a unique feature.
  - **Periplasmic Space:** A distinct and prominent space located between the inner (cytoplasmic) membrane and the outer membrane. Contains various proteins, including hydrolytic enzymes and transport proteins.
  - **Lipopolysaccharide (LPS):** Present in the outer leaflet of the outer membrane. LPS consists of Lipid A (endotoxin), core polysaccharide, and O antigen. It contributes to pathogenicity and provides a protective barrier.
  - **Sensitivity to Penicillin/Lysozyme:** Generally less susceptible due to the protective outer membrane, which acts as a barrier to many antibiotics and enzymes.

- **Examples:** *Escherichia coli*, *Salmonella*, *Pseudomonas*, *Neisseria*.

11. (b) Describes the process of specialized transduction in bacteria.

- Specialized transduction is a type of horizontal gene transfer in bacteria where a temperate bacteriophage (phage) transfers specific bacterial genes from one bacterium to another. This process occurs during the lysogenic cycle of a phage.
- **Steps of Specialized Transduction:**
  - **Integration of Prophage:** A temperate phage (e.g., lambda phage) infects a bacterium and integrates its DNA (now called a prophage) into a specific site on the bacterial chromosome. The integration site is usually adjacent to specific bacterial genes.
  - **Induction and Aberrant Excision:** Under certain stress conditions (e.g., UV radiation), the prophage is induced to excise from the bacterial chromosome and enter the lytic cycle. Occasionally, during this excision, an error occurs, and the phage genome excises imperfectly, taking with it a small piece of the adjacent bacterial DNA. Simultaneously, a small piece of the phage DNA is left behind in the bacterial chromosome.
  - **Packaging of Hybrid Phage Genome:** The newly formed phage particle, now called a transducing phage, contains a hybrid genome consisting of most of the phage DNA and a portion of the bacterial DNA that was adjacent to its integration site. Only bacterial genes located close to the phage integration site can be transduced.
  - **Infection of New Host:** This transducing phage then infects a new recipient bacterial cell.

- **Integration/Expression in New Host:** The hybrid phage genome can either integrate into the new host's chromosome (lysogeny) or enter a lytic cycle. If it integrates, the recipient bacterium acquires the specific bacterial genes carried by the transducing phage, along with the phage DNA. The newly acquired bacterial genes can then be expressed in the recipient cell.

- **Significance:** Specialized transduction allows for the transfer of a limited set of specific bacterial genes, which are always the ones located near the phage's integration site. It is a valuable tool in bacterial genetics for mapping genes and studying gene function.

12. (c) Discuss the life cycle of Candida albicans.

- *Candida albicans* is a dimorphic opportunistic pathogenic fungus that can exist in both yeast and hyphal forms. Its life cycle involves several stages, often dictated by environmental conditions within the host.

- **Key Stages in the Life Cycle:**

  - **Yeast Form (Budding):**

    - In its most common commensal state, *C. albicans* exists as a budding yeast.

    - Reproduction occurs asexually through budding, where a small outgrowth (bud) forms on the parent cell, enlarges, and eventually separates, forming a new daughter cell.

    - This is the prevalent form in normal microbiota (e.g., in the gut, oral cavity).

  - **Germination and Hyphal Formation:**

- Under specific environmental conditions (e.g., neutral pH, body temperature, presence of serum, nutrient availability), yeast cells can undergo a process called "germination."
- A germ tube, which is an elongated outgrowth, emerges from the yeast cell.
- The germ tube then elongates and develops into true hyphae (long, branching filaments) or pseudohyphae (chains of elongated yeast cells that resemble hyphae but have constrictions at septa).
- This transition from yeast to hyphal form is often associated with pathogenicity, as hyphae can invade tissues.

- **Invasion and Pathogenesis:**
  - The hyphal forms are particularly important for tissue invasion and biofilm formation.
  - They produce various virulence factors such as adhesins (for attachment to host cells), proteases, and phospholipases (for tissue damage).
  - The dimorphic switch is a key factor in the pathogenicity of *C. albicans*, allowing it to adapt to different host niches and evade immune responses.
- **Biofilm Formation:**
  - *C. albicans* can form robust biofilms on medical devices (e.g., catheters) and host surfaces.
  - Biofilms provide protection against antifungal agents and host immune cells.

- The formation of biofilms often involves the transition from yeast to hyphal forms, which contribute to the structural integrity of the biofilm.

▪ **Reversion to Yeast Form:**

- When conditions become less favorable for hyphal growth (e.g., nutrient limitation, altered pH), *C. albicans* can revert back to its yeast form, which is better suited for dissemination to new sites or for survival in harsh conditions.

▪ **Parasexual Cycle (Genetic Recombination):**

- While primarily asexual, *C. albicans* has been shown to undergo a parasexual cycle involving diploid cells undergoing mating, followed by mitotic recombination and chromosome loss, leading to genetic diversity. This is not a true meiotic sexual cycle but contributes to genetic variation.

- The ability of *C. albicans* to switch between yeast and hyphal forms is crucial for its survival, colonization, and pathogenic potential within the human host.

13. (a) Discuss the pathogenic effects of Staphylococcal infections that are caused by contaminated food.

- Staphylococcal food poisoning is a common foodborne illness caused by the ingestion of enterotoxins produced by certain strains of *Staphylococcus aureus* that have grown in contaminated food. It is an intoxication, not an infection, meaning the illness is caused by the preformed toxins, not by the growth of bacteria in the host.

- **Pathogenic Effects and Characteristics:**

▪ **Source of Contamination:** *S. aureus* is commonly found on human skin, in nasal passages, and on hair. Food

contamination often occurs when food handlers do not wash their hands properly or when they handle food while having skin infections (e.g., boils, cuts).

- **Toxin Production:** *S. aureus* produces heat-stable enterotoxins (e.g., SEA, SEB, SEC) when it multiplies in food. These toxins are not destroyed by cooking, even if the bacteria themselves are killed.
- **Food Vehicles:** Foods commonly associated with staphylococcal food poisoning include those that are handled extensively and not reheated after preparation, such as custards, cream-filled pastries, potato salad, chicken salad, ham, and other cooked meat products.
- **Rapid Onset of Symptoms:** Symptoms typically appear rapidly, usually within 1 to 6 hours (often 2-4 hours) after ingesting the contaminated food, due to the preformed toxins.
- **Gastrointestinal Symptoms:** The primary effects are on the gastrointestinal tract.
    - **Nausea and Vomiting:** Often severe and sudden, leading to rapid evacuation of stomach contents. This is a hallmark symptom.
    - **Abdominal Cramps:** Intense pain in the abdomen.
    - **Diarrhea:** May or may not be present; if present, it is usually mild to moderate.
    - **Prostration:** A feeling of extreme weakness or exhaustion.
    - **Headache and Sweating:** May also occur.

- **Short Duration:** The illness is usually self-limiting and relatively short-lived, typically resolving within 24 to 48 hours as the toxins are expelled from the body.
- **No Fever:** Fever is generally absent or low-grade, which helps differentiate it from other foodborne infections.
- **No Person-to-Person Spread:** Since it's an intoxication, it's not typically transmitted directly from person to person.

- Preventive measures focus on proper food handling, refrigeration, and hygiene to prevent *S. aureus* growth and toxin production in food.

14. (a) Discuss the structure of Influenza virus. Discuss the measures to control its spread.

- **Structure of Influenza Virus:**

- Influenza viruses are enveloped, single-stranded RNA viruses belonging to the Orthomyxoviridae family. Their structure is critical for their infectivity and immune evasion.
- **Genome:** Consists of 8 segmented negative-sense single-stranded RNA (ssRNA) molecules. Each segment typically encodes one or two proteins. This segmented genome allows for genetic reassortment (antigenic shift).
- **Nucleocapsid:** Each RNA segment is associated with nucleoproteins (NP) and RNA-dependent RNA polymerase (RdRp) enzymes (PB1, PB2, PA), forming ribonucleoprotein (RNP) complexes. These RNP complexes are coiled into a helical nucleocapsid.
- **Matrix Protein (M1):** A layer of matrix protein (M1) underlies the viral envelope, providing structural integrity and linking the nucleocapsid to the envelope.

- **Viral Envelope:** A lipid bilayer derived from the host cell membrane during budding. Embedded within the envelope are two major surface glycoproteins:
    - **Hemagglutinin (HA):** A trimeric glycoprotein responsible for binding to sialic acid receptors on host cells, initiating infection. It is also the primary target for neutralizing antibodies. There are 18 known HA subtypes.
    - **Neuraminidase (NA):** A tetrameric glycoprotein that cleaves sialic acid residues, facilitating the release of newly formed virions from infected cells and preventing self-aggregation. There are 11 known NA subtypes.
- **Ion Channel (M2 Protein):** In influenza A, a small ion channel protein (M2) is present in the envelope. It plays a role in uncoating by allowing protons into the virion core, lowering the pH and facilitating the dissociation of the RNP complexes from M1.

- **Measures to Control its Spread:**
    - **Vaccination:**
        - Annual influenza vaccination is the most effective way to prevent influenza and its severe complications. Vaccines are formulated each year based on predictions of circulating strains (trivalent or quadrivalent vaccines).
        - Reduces the risk of infection, severity of illness, hospitalization, and death.
    - **Antiviral Medications:**

- Antiviral drugs (e.g., oseltamivir, zanamivir, peramivir, baloxavir) can treat influenza by inhibiting viral replication.
- Most effective when started within 48 hours of symptom onset.
- Can also be used for prophylaxis (pre-exposure or post-exposure).

- **Hand Hygiene:**
  - Frequent and thorough handwashing with soap and water or using alcohol-based hand sanitizers reduces the spread of respiratory droplets containing the virus.
- **Respiratory Etiquette:**
  - Covering coughs and sneezes with a tissue or into the elbow, rather than hands, helps contain respiratory droplets.
  - Prompt disposal of used tissues.
- **Avoid Touching Face:**
  - Avoid touching eyes, nose, and mouth to prevent the transfer of the virus from contaminated surfaces to mucous membranes.
- **Social Distancing:**
  - Maintaining physical distance from individuals who are sick can reduce exposure to respiratory droplets.
  - Avoiding large gatherings during peak influenza season.

- **Isolation of Sick Individuals:**
    - People with influenza symptoms should stay home from work, school, or public places to prevent further transmission.
- **Cleaning and Disinfection:**
    - Regular cleaning and disinfection of frequently touched surfaces (e.g., doorknobs, countertops) can help reduce viral survival on surfaces.
- **Surveillance and Monitoring:**
    - Public health surveillance systems monitor influenza activity and identify new or emerging strains, which informs vaccine development and public health responses.

15. (b) Expand the following acronyms (any five):
    - (i) SEM
        - Scanning Electron Microscopy (or Microscope)
    - (ii) NAG
        - N-Acetylglucosamine
    - (iii) MPN
        - Most Probable Number
    - (iv) Hfr
        - High Frequency of Recombination
    - (v) FISH
        - Fluorescence In Situ Hybridization
    - (vi) CFU

        - Colony-Forming Unit
    - (vii) VBNC
        - Viable But Non-Culturable

16. (c) Provide a concise explanation of the one-step growth curve in virus multiplication within a host cell, and include suitable diagrams to illustrate the process.

    - **Explanation of the One-Step Growth Curve:**
        - The one-step growth curve describes the kinetics of virus replication in a synchronized population of host cells following a single, initial infection event. It typically divides the viral replication cycle into distinct phases:
            - **Eclipse Phase:**
                - Starts immediately after the virus infects the host cell.
                - During this phase, the virus uncoats, and its genetic material takes control of the host cell's machinery to synthesize viral components (proteins, nucleic acids).
                - No infectious viral particles can be detected extracellularly or intracellularly during this period, as the virus is disassembled.
            - **Maturation/Latent Phase:**
                - Follows the eclipse phase.
                - Viral components are assembled into new, infectious virions inside the host cell.
                - The number of infectious intracellular virions begins to rise, but they are not yet released from the cell. The total number of infectious

virions (intracellular and extracellular) remains low.

- **Release Phase (Burst Phase):**
    - New virions are released from the host cell.
    - For lytic viruses, this involves cell lysis, leading to a sudden, sharp increase in the number of infectious virions in the extracellular environment.
    - For enveloped viruses, this often occurs through budding, a more gradual process.
    - The "burst size" refers to the average number of new infectious virions produced per infected cell.

- The curve gets its name because the viral population increases in a single "step" or burst after the initial lag, rather than a continuous exponential increase.

- **Diagram (Conceptual Description - Not actual drawing):**
    - Imagine a graph with "Time after infection" on the X-axis and "Log of Infectious Virions" on the Y-axis.
    - **Phase 1 (Eclipse Phase):** The curve starts at a baseline (initial input virus) and then rapidly drops to zero (or very low levels) as viruses uncoat. This phase shows no increase in infectious virions.
    - **Phase 2 (Latent Phase):** The curve remains flat and low for a period, representing the time when viral components are being synthesized and assembled, but not yet released.
    - **Phase 3 (Burst/Release Phase):** The curve sharply rises, indicating the rapid release of new infectious virions

from the host cells, reaching a plateau at the maximum viral titer.

17. (b) Describe Robert Koch's postulates describing the relationship between microorganisms and the disease caused.

- Robert Koch's postulates are a set of four criteria designed to establish a causal relationship between a specific microorganism and a specific disease. They were formulated in the late 19th century and played a crucial role in the development of microbiology.
- **Koch's Postulates:**
  - **1. The microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms.**
    - This means that the suspected pathogen should be consistently associated with the disease.
  - **2. The microorganism must be isolated from a diseased organism and grown in pure culture.**
    - The pathogen needs to be separated from other microorganisms and cultivated outside the host in an artificial medium.
  - **3. The cultured microorganism should cause disease when introduced into a healthy organism.**
    - Introducing the pure culture of the suspected pathogen into a susceptible, healthy host should reproduce the original disease symptoms.
  - **4. The microorganism must be re-isolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent.**

- The pathogen must be recovered from the experimentally infected host and confirmed to be the same microorganism as the one originally isolated.

- **Limitations of Koch's Postulates:**
  - While foundational, these postulates have some limitations in modern microbiology:
    - **Asymptomatic Carriers:** Some pathogens can exist in asymptomatic carriers who do not show disease symptoms but can transmit the pathogen (e.g., *Salmonella Typhi* in typhoid carriers).
    - **Unculturable Microorganisms:** Some microorganisms cannot be grown in pure culture outside the host (e.g., *Treponema pallidum* for syphilis, *Mycobacterium leprae* for leprosy, viruses).
    - **Polymicrobial Diseases:** Some diseases are caused by multiple pathogens, or involve a complex interplay of microorganisms.
    - **Opportunistic Pathogens:** Microorganisms that are part of the normal flora can cause disease only when the host's immune system is compromised.
    - **Ethical Concerns:** It is unethical to intentionally infect human volunteers with deadly pathogens for research.

18. (c) Write short notes on (any four):

- (i) Capsids
  - Capsids are the protein coats that enclose the nucleic acid (DNA or RNA) of a virus. They are made up of repeating protein subunits called capsomeres. The capsid

protects the viral genome from physical, chemical, and enzymatic degradation. They also play a crucial role in viral attachment to host cells and, in some cases, in the penetration of the host cell. Capsids can exhibit various symmetrical shapes, including helical, icosahedral, or complex structures, which are characteristic of different virus families.

- (ii) Lipopolysaccharides (LPS)
  - Lipopolysaccharides (LPS) are complex molecules found exclusively in the outer membrane of gram-negative bacteria. They are also known as endotoxins because they are an integral part of the bacterial cell wall and are released upon cell lysis, causing various pathological effects in the host. LPS consists of three main parts: Lipid A (the toxic component), a core polysaccharide, and an O-antigen (a highly variable polysaccharide chain that is recognized by the host immune system). LPS contributes to the structural integrity of the outer membrane and acts as a potent immunostimulant, triggering strong inflammatory responses, which can lead to fever, shock, and even death in severe infections.
- (iii) Chemotaxis
  - Chemotaxis is the directed movement of a motile cell or organism in response to chemical stimuli in its environment. In bacteria, it refers to the ability to sense and move towards attractants (e.g., nutrients like sugars and amino acids) or away from repellents (e.g., toxic substances). Bacteria achieve chemotaxis by modulating their flagellar movement, alternating between "runs" (straight movement towards an attractant) and "tumbles" (random reorientation). This directed movement is crucial for bacteria to locate favorable environments and avoid

harmful ones, thus contributing to their survival and pathogenesis.

- (iv) Inclusion Bodies
    - Inclusion bodies (or storage granules) are discrete aggregates of various substances found within the cytoplasm or periplasm of bacterial and archaeal cells. They are typically composed of reserve materials, such as carbon, energy, or structural building blocks, accumulated when nutrients are abundant and utilized when environmental conditions are less favorable. Examples include polyhydroxybutyrate (PHB) granules (for carbon and energy storage), glycogen granules, polyphosphate granules (volutin granules, for phosphate storage), and sulfur granules. Inclusion bodies are generally non-membrane bound, though some may be enclosed by a single layer of protein. They allow cells to store essential resources efficiently without increasing osmotic pressure.

- (v) Classification of viruses on the basis of nucleic acids
    - Viruses are classified based on the type of nucleic acid they possess, a fundamental criterion in virology. The Baltimore classification system is a widely used method that categorizes viruses into seven groups based on their genome type and replication strategy. The main categories based on nucleic acids are:
        - **DNA Viruses:**
            - Double-stranded DNA (dsDNA) viruses (e.g., Herpesviruses, Poxviruses)
            - Single-stranded DNA (ssDNA) viruses (e.g., Parvoviruses)

- **RNA Viruses:**
    - Double-stranded RNA (dsRNA) viruses (e.g., Reoviruses)
    - Positive-sense single-stranded RNA (+ssRNA) viruses (e.g., Picornaviruses, Flaviviruses)
    - Negative-sense single-stranded RNA (-ssRNA) viruses (e.g., Orthomyxoviruses, Rhabdoviruses)
- **Reverse-Transcribing Viruses:**
    - Double-stranded DNA reverse-transcribing (dsDNA-RT) viruses (e.g., Hepadnaviruses - Hepatitis B virus)
    - Single-stranded RNA reverse-transcribing (ssRNA-RT) viruses (e.g., Retroviruses - HIV)

- This classification helps understand viral replication mechanisms and development of antiviral strategies.

- (vi) Organisms not seen on Gram Stain
    - While Gram staining is a cornerstone technique in microbiology, certain microorganisms either do not stain effectively or do not possess the peptidoglycan-based cell wall structure that the Gram stain targets.
        - **Mycoplasmas:** Lack a cell wall entirely, so they do not retain crystal violet and appear unstained.
        - **Mycobacteria:** Have a unique waxy cell wall rich in mycolic acids, which prevents the uptake of crystal violet. They are "acid-fast" and require specific

staining methods like the Ziehl-Neelsen stain (e.g., *Mycobacterium tuberculosis*).

- **Spirochetes:** Are very thin and long, making them difficult to visualize with Gram stain. Dark-field microscopy or silver staining methods are typically used (e.g., *Treponema pallidum*).
- **Rickettsiae and Chlamydiae:** These are obligate intracellular bacteria that have complex life cycles and are too small or their cell wall structure is too unique for effective Gram staining. They are usually visualized using specialized stains or electron microscopy.
- **Fungi:** While some fungi may stain weakly Gram-positive, they are eukaryotic and have cell walls made of chitin, not peptidoglycan, so Gram staining is not appropriate for their identification. Special fungal stains are used.
- **Viruses:** Viruses are not cells and do not have cell walls, so they cannot be Gram-stained.