The Pokemon Mindset (W1)

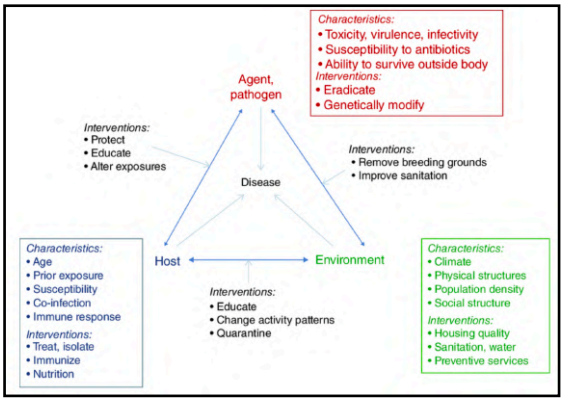
Microbes (L1)

Living with microbes

- Humans live with trillions of microorganisms
- The estimated total number of bacteria in a 70kg man is 3.8 x 10^13
- Ratio of microbial to human cells for the average man ~ 1.3:1
- Microbes are everywhere

Concepts of disease occurrence

- A microorganism found on/in a host may be:
  - A **commensal**
  - A **pathogen**
  - An **opportunistic pathogen**
- Agent/pathogen characteristics
  - **Toxicity, virulence, infectivity**
  - **Susceptibility** to antibiotics
  - Ability to **survive** outside body
  - **Interventions**: eradicate, genetically modify
- Environment characteristics
  - Climate, **physical** structures, population density, social structure
  - **Interventions**: house quality, sanitation, water, preventive services
- **Host** characteristics
  - Age, prior exposure, susceptibility, co-infection, immune response
  - Interventions: treat, isolate, immunise, nutrition
- Pathogen and environment interventions: remove breeding grounds, improve sanitation
- Environment and host interventions: educate, change activity patterns, quarantine
- Host and pathogen interventions: protect, educate, alter exposure
- Pathogen + Environment + Host —> Disease



Commensal

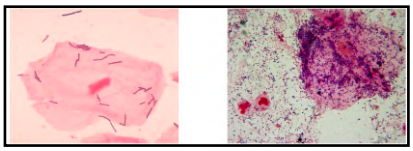
- When **two or more organisms coexist where one benefits and the other derives neither benefit nor harm**, the process is called commensalism. Such an organism is referred to as a commensal
- Commensalism typically involves bacteria; however, some fungi, viruses and protozoa can also coexist with the human host
- **Mutualism -** where **both the commensal and the host benefit from the relationship**
- Commensal microflora also known as **normal microflora**, is a complex ecosystem composed by these resident bacteria

The commensal ecosystems in the human body

- **Heavily colonised** body sites. These sites harbour large numbers of commensal organisms
  - **Oral cavity, nasopharynx, bowel, vagina, skin**
- **Sterile** body sites: no commensals (or other) bacteria are normally present in these sites
  - **Blood stream, cerebrospinal fluid, bladder, peritoneal space, joint space**, etc

Commensal example

- Gram stain from **healthy vagina** showing predominant Gram-positive bacilli (Lactobacillus) and large pink squamous epithelial cell
- Gram stain from vagina with bacterial vaginosis. Loss of lactobacilli and **overgrowth** of **other commensals** including Gram-variable coccobacilli (Gardnerella vaginalis), many covering an epithelial cell
- ***Lactobacilli* produce lactic acid from lactose, thus maintaining the pH of the vagina at 3.8-4.5**
- This is optimal for the **right balance of normal vaginal commensals**



Pathogens
- Microorganisms **capable of causing disease in a host**
- May be:
  - Exogenous or endogenous
- Note: some pathogens may exist in a **carrier state** (carriage), where they **coexist with normal flora without causing disease**. However, under certain conditions this can lead to infections
- **Endogenous** infection: arises from the **host's own commensal flora**
  - e.g. bacteria from a ruptured bowel causing peritonitis
- **Exogenous** infection: originates from an **external source**
  - e.g. influenza virus transmitted from another person

Examples of pathogen carriage
- Examples of **pathogen carriage**:
  - ***Neisseria meningitidis* population** (nasopharynx) (10%)
  - ***Streptococcus pneumoniae*** (nasopharynx) (5-70%)
  - ***Staphylococcus aureus*** (nose, skin) (25-30%)
- Some pathogens may be carried following recovery from disease:
  - e.g. ***Salmonella Typhi*** may persist in the faeces for months following recovery from disease
  - Asymptomatic carriers can serve as a source of infection for others

Virulence factors
- Characteristics of a microorganism that **increase the ability of that organism to cause disease**
- Evasion of host defences, sequestration in an inaccessible site, produce a toxin, resistance to antimicrobial agents

Category	Virulence factor example	Correlating microorganism example
Evasion of host defences	M protein evade phagocytosis by down regulating complement and interfering with opsonization.	*Streptococcus pyogenes*
Sequestration in an inaccessible site	Intracellular location in macrophages	*Mycobacterium tuberculosis*
Production of toxin	Blocking release of neurotransmitter acetylcholine from nerve endings	*Botulinum toxin (Clostridium botulinum)*
Resistance to antimicrobial agents	Resistance to antiviral agents	Human immunodeficiency virus

Opportunistic pathogens
- Microorganisms with **low virulence** that are **typically harmless** as part of the **body's commensal flora** but can **cause disease under certain conditions**, such as:
  - **Breakdown** in host's innate **immune defences**
  - Host immune defences are impaired (e.g. **immunocompromised** patients)
  - Example: ***Staphylococcus epidermidis*** is part of the skin flora. When introduced into the bloodstream, e.g. via a peripherally inserted central catheter (PICC), it can cause **infective endocarditis**, particularly in the presence of prosthetic heart valves

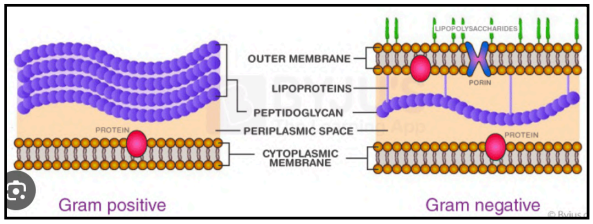
Classes of infectious agents
- Fungi and parasites (eukaryotic)
- Bacteria (prokaryotic)
- Viruses and prions

Prokaryotic vs Eukaryotic cells
- **Prokaryotic**
  - Genome consisting of a **single chromosome**
  - **No nuclear membrane** around genome
  - **No membrane bound organelles**
  - Small (0.1-5μm)
  - Example: bacteria and mycobacteria
- **Eukaryotes**
  - **Multiple** linear chromosomes
  - Genome **enclosed in nucleus**
  - Contains **organelles** (e.g. mitochondria and endoplasmic reticulum)
  - Larger (10-100μm)
  - Example: human, fungi and parasites

Taxonomy
- Applicable to bacteria, fungi and parasites
- A **way of organising organisms according to their phenotypic and genetic properties**

- Grouped according to similarities and differences. Example:
  - Domain: bacteria
  - Phylum: Proteobacteria
  - Class: delta-proteobacteria
  - Order: enterobacteriales
  - Family: enterobacteriaceae
  - **Genus**: **Escherichia**
  - **Species**: **coli**
- Similarity increases as you go down

<u>Bacteria</u>



- Prokaryotic: bacteria are **single-celled** prokaryotic organisms without a nucleus
- Essential structures:
  - **Cell wall**: provides **structural support and shape**
    - Gram positive (purple): bacteria have a **thick peptidoglycan layer**
    - Gram negative (pink): bacteria have a **thin peptidoglycan layer and an outer membrane** containing **lipopolysaccharides** (LPS)
  - **Cytoplasm**: contains **ribosomes** and essential cellular components
  - Nuclear material: DNA exists as a **single circular chromosome**, not enclosed within a nucleus (plasmid)
- Special structures (not present in all bacteria)
  - **Capsule**: protective outer layer that prevents phagocytosis
  - **Flagella**: provides motility
  - **Pili**: provides motility
  - **Spores**: allow bacteria to survive harsh environments (e.g. Bacillus and Clostridium species)

<u>Fungi</u>
- Eukaryotic: fungi have a **defined nucleus** and **membrane-bound organelles**
- Forms of fungi: can exist as **single-celled** organisms (yeast) or **multicellular** organisms (moulds)
- Key characteristics:
  - Cell structure: **thick cell wall** containing **chitin** (polysaccharide, fibrous)
- Reproductive structures:
  - **Yeast**: reproduce by **budding or fission** (e.g. *Candida sap* and *Malassezia*)
  - **Moulds**: form conidia (**spores**) (e.g. *Penicillium* spp)

<u>Parasites</u>
- Parasites are eukaryotic microbes with vast diverse forms
- Structural variability:
  - **Single-celled** organisms such as **protozoa**
  - Complex **multicellular** organisms with specialised organs and tissues, including GI and reproductive systems e.g. worms
- **Life cycles**: parasites often have intricate life cycles, progressing through multiple developmental stages. These stages may require one two intermediate hosts before reaching and infecting the definitive host
- **Geographic and environmental niche**: many parasitic diseases are geographically specific
  - Diagnosis and management require knowledge of travel history, lifestyle factors, pet ownership, and potential environmental exposures

<u>Viruses</u>
- Extremely small, typically ranging from 20 to 400nm
- Contain **DNA** or **RNA** enclosed within a **protein shell** called a **capsid**
- Some viruses also possess a **lipid envelope** surrounding the capsid
- Lack of independent functionality:
  - **Lack** the necessary **biochemical organelles or processes** to propagate **independently**
  - Must infect a host organism to replicate and sustain their lifecycle

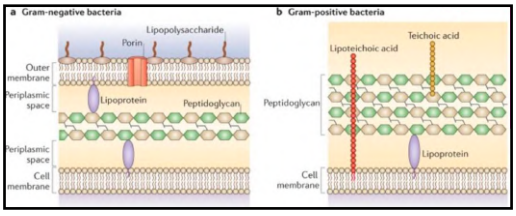
Prions
- Prions do **not contain DNA or RNA**, differentiating them from viruses and other microorganisms
- They cannot replicate in the traditional sense
- Prions are **misfolded proteins** capable of **inducing** other **normal proteins to adopt the same abnormal structure**
- This process leads to a self-propagating replicative cycle
- Non-living entities: prions are not considered organisms as they lack cellular structures and metabolic processes
- Associated disease: responsible for **fatal neurodegenerative disorders**, such as Creutzfeldt-Jakob disease (CJD). These diseases result from the **accumulation of abnormal prion** proteins in the brain
- Resistance to inactivation: prions are highly resistance to standard sterilisation methods, making them challenging to eliminate

Diagnostic microbiology
- History: 56%
- Examination: 17%
- Other: Lab tests and imaging: 27%

Sample type considerations
- Diagnosis is based on the **detection from relevant clinical specimens** based on clinical presentation
- **Fresh** specimens are better
- **Adequate volume**
- **Aseptic** and additional procedure to **avoid contaminating** specimen and protecting yourself

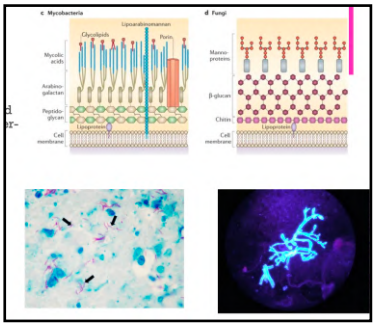
Specimen types in microbiology
- **Sterile container**: used for collecting sputum, urine or stool specimens
- **Black swab**: swab with charcoal transport mediate to ensure **viability of bacteria** during **transit**
- **White swab**: dry swab for **nucleic acid amplification** test (e.g. PCR)
- **Flocked swab with viral transport media**: Flocked structure **enhances absorption** and **release of sample** used for collecting specimens from upper **respiratory tract for nucleic acid amplification test** (e.g. PCR) of **viral** infections
- **Blood sample in EDTA tube**: used of thick and thin films, nucleic acid amplification test (e.g. PCR)
  - EDTA **prevents** sample from **clotting**
- **Blood sample in SST tube**: used for **serological tests**. Contain **clot activator** to facilitate serum separation
- **Blood cultures**: used for detecting **bacteraemia**. Contains an indicator to detect carbon dioxide from bacteria metabolism

Microscopy - Bacteria: Gram stain
- Most clinically significant bacteria can be observed under a microscope at 1000x magnification
- Bacteria are classified into two broad groups based on their cell wall structure, identified using the Gram stain:
  - Gram-positive bacteria appear blue/purple due to their **thick peptidoglycan cell wall**
  - Gram-negative bacteria appear pink/red due to their **thinner peptidoglycan cell wall** and **outermembrane**



- The next step involves identifying the **shape** of the bacteria, such as **cocci** (spherical) or **bacilli** (rod-shaped)
- Understanding Gram stain results and bacterial morphology is crucial as they are often the first details provided by the laboratory after a specimen is submitted

Mycobacteria and fungi



- Mycobacteria:
  - Mycobacteria have **thick, lipid-rich mycolic acid cell walls** that make then **resistance** to many water-based stains
  - Ziehl-Neelsen technique uses carbon fuchsin (lipid-soluble) combined with heat to penetrate the cell wall
- Fungi:
  - Calcofluor White binds to **chitin**, a **structural polysaccharide** in fungal **cell walls**, making it a reliable marker as it is absent in human cells
  - Under **UV light**, fungi fluoresce blue or white, enhancing their visibility even in dense or complex specimens

Culture

- **Purpose**: culture is essential for **identifying pathogenic organisms**, **characterising** them further, and determining **antibiotic susceptibility**
- Media types:
  - **Non-selective media** (e.g. blood agar, chocolate agar) support the **growth of a wide range** of organisms
  - **Differential media** (e.g. MacConkey agar) allows the **growth of multiple organisms** but contains substances that help **distinguish** between different organisms based on their characteristics
  - **Selective media** (e.g. Lowenstein-Jensen, Sabaroud agar) promote **specific organisms growth** while inhibiting others
- **Growth conditions**: optimal temperature and gas mixtures are critical. Organisms may require **aerobic**, **anaerobic**, **microaerophilic** (low oxygen) or **capnophilic** (high CO2) environments
- **Automation**: automated systems, like blood culture machines, **monitor CO2 production** by growing organisms
- **Timeframe**: cultures for most pathogens require **24-48 hours** for growth. There are **fastidious** organism that require **longer incubation** time

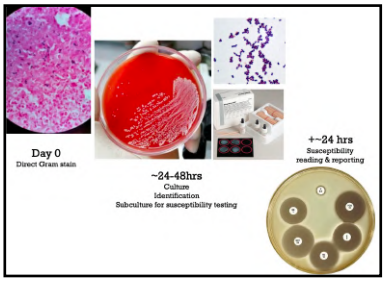
Phenotypic identification

- Essential for **confirming bacterial species** and guiding effective **clinical interventions**
- **Rapid benchtop tests**, such as latex agglutination (e.g., for *Staphylococcus aureus*), deliver quick results for commonly encountered pathogens
- **Biochemical methods** (e.g. API) rely on detecting the **metabolic properties of bacteria**. Organisms are incubated with **specific substrates**, leading to observable changes such as colour shifts, indicating **substrate utilisation** or **enzymatic activity**
- MALDI-TOF MS represents advanced diagnostic technology, analysing a **bacterium's protein fingerprint** to generate a **unique mass spectrum** that is compared to a **database** for species identification

Susceptibility test

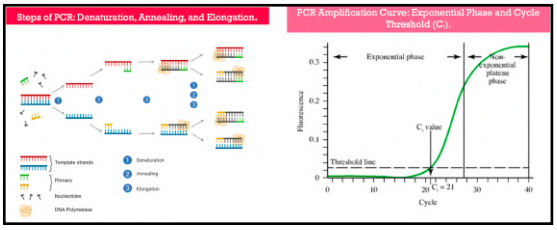
- Susceptibility testing assesses the **effectiveness of antimicrobial agents** against pathogens and is performed to guide therapeutic decisions and monitor resistance trends
- Methods of Susceptibility Testing:
  - **Disk Diffusion** (Kirby-Bauer Method): Involves placing **antibiotic-impregnated disks** on an **agar plate** inoculated with the **microorganism**. The size of the **zone of inhibition** indicates the organism's susceptibility
  - **E-Test**: Combines the principles of **disk diffusion and MIC** (Minimum Inhibitory Concentration) determination. An antibiotic **gradient strip** is placed on an agar plate to **determine the MIC value**.
  - **Automated Systems**: Systems such as VITEK and BD Phoenix rapidly perform susceptibility testing and provide detailed MIC results
- Limitations
  - Requires a **live organism** and established breakpoints for interpretation.
  - **Emerging resistance** mechanisms may necessitate alternative detection methods (e.g., molecular diagnostics).
  - Results can be misinterpreted if clinical context is not considered

Example of a report



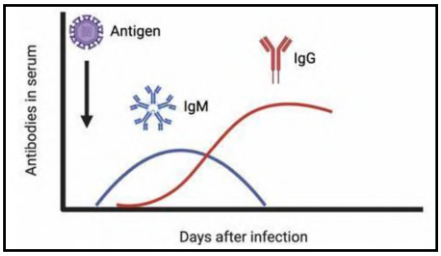
- Wound swab (first)
  - Leukocytes: many
  - Bacteria: **Gram positive cocci** seen
- Culture (second)
  - Abundant growth of ***Staphylococcus aureus***
- **Antibiotics** (third)
  - Penicillin R; Flucloxacillin S; Cephalaxin S; Clindamycin S

Nucleic acid amplification test



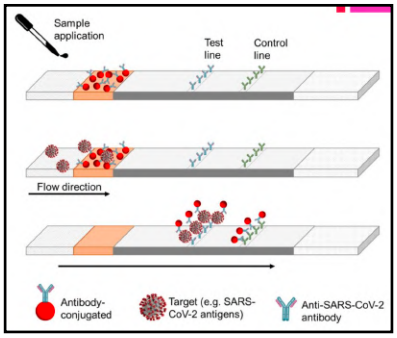
- Polymerase chain reaction
- Steps of PCR: **Denaturation** (split DNA up), **annealing** (match **primer**) and **elongation** (nucleotides and DNA polymerase)
- PCR amplification curve: exponential phase and cycle threshold (Ct - how many cycles needed to detect organism)
- PCR is a rapid and sensitive method for detecting the DNA or RNA of a specific microbial target

Serology

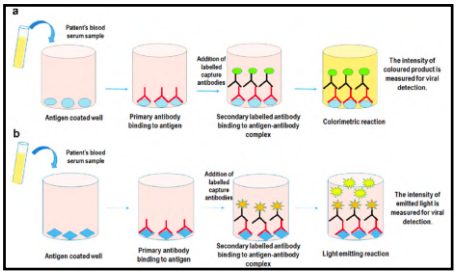


- Serology detects **antigens** to identify infections or **antibodies** to assess **immune responses** to infections or vaccinations
- General types of targets
  - **Antigens**: Components of pathogens (e.g., capsule), useful for detecting infections in early stages before antibodies are produced
  - **IgM Antibodies**: Appear first, indicating an **early or acute phase** of infection
  - **IgG Antibodies**: Develop **later**, signifying **long-term immunity** or **past exposure** and **recovery**
- Limitations
  - **Time dependent**: **Antigen** detection is effective only during the **acute phase of infection**, while **antibody** detection requires a **convalescent (recovering) serum** to detect seroconversion
  - **Cross reactivity**: Notably observed with **IgM** due to the **immaturity of its binding site**, which **limits specificity and affinity**

Serology - **antigen** detection

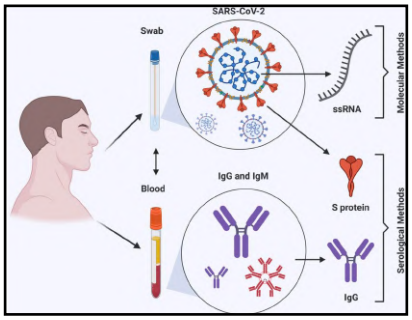


- Antigen detection utilises **specific antibodies to bind and identify pathogen-associated antigens**, such as those from SARS-CoV-2, on a testing strip. The **antigen-antibody** interaction produces a **visible signal** at the test line, with a control line confirming test validity
- Role in Rapid Diagnosis
  - Provides quick results, often within 15–30 minutes, enabling immediate decision-making.
  - Particularly useful in point-of-care settings, mass testing, or during outbreaks.
  - Ideal for detecting active infections in symptomatic patients
- Limitations
  - Sensitivity: **Lower sensitivity** compared to molecular methods like RT -PCR, especially in low viral load cases or early/late stages of infection.
  - Specificity: Potential for **false positives** due to **cross-reactivity with other antigens** or improper handling.
  - Open to user interpretation



Serology - **antibody** detection

- Antibody detection involves identifying specific antibodies (e.g., IgM or IgG) in a patient's serum by **capturing them on antigen-coated wells**. The presence of antibodies is detected using **labelled secondary antibodies** that produce a **measurable signal**, such as a **colour** change or light emission, proportional to antibody levels
- Use of Antibody Detection:
  - Helps confirm **past exposure** to pathogens or **assess immune response post-vaccination**
  - Essential for seroprevalence studies to measure the spread of infection in populations
  - Often used in conjunction with other diagnostic tests to provide a complete picture of infection status
- Limitations:
  - **Time Dependency**: Requires a convalescent serum sample, as antibodies may not be detectable during the early stages of infection
  - **False Positives**: Cross-reactivity with antibodies to related pathogens can reduce specificity
  - **False Negatives**: Variability in immune responses or low antibody levels can lead to undetected cases
  - Cannot distinguish between active and resolved infections



COVID example

- Immunochromatography test (ICT):
  - I have done a RAT and I have COVID
- Nucleic acid amplification test result:
  - SARS-CoV-2 RNA: detected
- Serology performed 3 weeks later:
  - SARS-CoV-2 S protein IgG (CMIA): Detected
  - SARS-CoV-2 N protein IgG (CMIA): Detected
  - Consistent with recent or past SARS-CoV-2 infection

Further characterisation - serotyping

- **Serotyping** identifies **bacterial strains** by detecting specific **surface antigens**, such as the O (lipopolysaccharide) and H (flagellar) antigens, using **agglutination reactions with antisera**; for example, in Salmonella, different serotypes are distinguished based on their unique combinations of these antigens.
- Enables further characterisation of organisms to **differentiate between species and serotypes**, such as *Salmonella Typhi*, which causes **typhoid fever**, and *Salmonella Enteritidis*, which is associated with **gastroenteritis**
- Serotyping requires specialised scientists, relies on the maintenance of high-quality antisera available only in reference laboratories. Viable organisms are necessary for testing

Further characterisation - sequencing

- First generation -> second generation (next generation segueing) -> third generation
- **First gen**: infer nucleotide using dNTPs then visualise with electrophoresis, 500-1000 bp fragments
- **Second gen**: high throughput from the parallelisation of sequencing reactions, 50-500 bp fragments
- **Third gen**: sequence native DNA in real time with single-molecule resolution, tens of kb fragments, on average
- Sequencing determines the **complete or partial nucleotide sequence** of an organism's DNA or RNA.
- Enable **precise identification and differentiation** of pathogens, tracks outbreaks through phylogenetic analysis, detects **antimicrobial resistance genes**, and supports vaccine development and research into pathogen evolution.
- Sequencing requires specialised equipment, skilled personnel, and advanced bioinformatics tools, making it resource-intensive

Summary

- Disease caused by infectious agents is influenced by the interaction between the host, the pathogen, and environmental factors.
- The five main types of clinically significant infectious agents include bacteria, fungi, parasites, viruses, and prions.
- A wide variety of specimens are necessary for microbiological diagnostics and collection, transport, and processing are critical for accurate diagnostic outcomes.
- Discussed the principles, advantages, and limitations of diagnostic techniques such as microscopy, culture, molecular, and serological methods, using examples like wound swabs and COVID testing.
- Explored organism characterisation, including antimicrobial susceptibility testing, serotyping, and sequencing methodologies

Antibiotics (W3)

The Berlin Wall (L1)

Definitions

- **Antimicrobials/Anti-infectives**: Drugs active **against microorganisms**, including antibacterials, antivirals, antifungals, and antiparasitic agents.
- **Antibiotics**: Natural or synthetic compounds that **inhibit bacterial biochemical functions** (e.g., cell wall synthesis, protein synthesis, DNA replication, or cellular respiration).
- **Antiseptics/Disinfectants**: Antimicrobial substances **too toxic for internal use**, used **externally** to kill or inhibit microorganisms.
- **Broad Spectrum Antibiotics**: Effective against a wide variety of bacterial species.
- **Narrow Spectrum Antibiotics**: Target specific bacterial species while minimising collateral damage to the microbiome and other commensal bacteria that coexist with us.

Types of antibiotics

- **Bactericidal**: kill organisms
- **Bacteriostatic**: inhibit organisms growth long enough for body defences to remove it

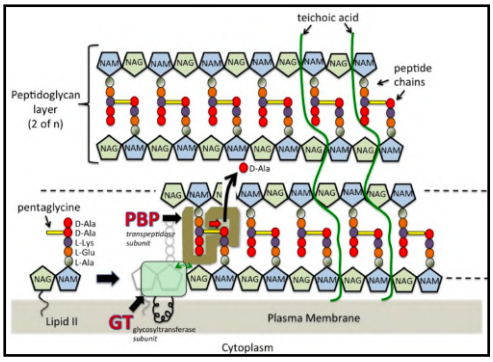
Antibiotic mechanisms of action

- **Cell wall synthesis**
  - Mechanism of action: inhibit bacterial cell wall synthesis, disrupting structural integrity and lead to cell death
  - **Beta-lactam** antibiotics - inhibit PBPs:
    - Penicillins
    - Cephalosporins
    - Monobactams
    - Carbapenems
  - **Glycopeptide** antibiotics - prevent transpeptidase binding:
    - Vancomycin
    - Teicoplanin
- **Folic acid metabolism** - folic acid is needed for nucleic acid synthesis
- Cell membrane
- **DNA replication** (DNA gyrase)
- DNA-dependent RNA polymerase
- **Protein synthesis** (50S inhibitor)
- **Protein synthesis** (30S inhibitor)

Cell wall

- Gram-negative bacteria:
  - **Thin peptidoglycan layer**
  - **Outermembrane with LPS**
- Gram-positive bacteria:
  - **Thick peptidogyclan layer**
  - No outermembrane
  - Teichoic acid

Cell wall structure



- Composed of a **peptidoglycan layer** made up of **repeating N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) subunits**
- **NAM subunits** have **short peptide chains (D-Ala to L-Ala)** attached that provide structural integrity by forming **cross-links** between **NAM subunits**
- **Penicillin-binding proteins (PBPs) catalyse** the **cross-linking of peptide chains** and are crucial for the final stages of peptidoglycan assembly
- **Teichoic acid** found in **Gram-positive bacteria** contribute to **cell wall stability** and bacteria **attachment** to mucosal surface