NATIONAL OPEN UNIVERSITY OF NIGERIA

# NSC 106

## Medical Mircobiology and Parasitology

## Module 4

# NSC 106 (Medical Microbiology and Parasitology) Module 3

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# Module 3: Introduction

Infection control is a core duty of all hospital workers that are directly involves in the care of patients. Nurses are faced with infection and infection control challenges in their daily practice. This module will deals with bacterial nutrition, growth and control.

At the end of this module, you should be able to discuss bacterial nutrient, its growth and how it can be controlled.

# Unit 1 Bacterial Nutrition and Growth

## 1.0 Introduction

Microbial cells are structurally complex and carry out numerous functions. Nutrients are required as materials that are used in biosynthesis and to make energy available. The growth of microorganisms depends upon an adequate supply of nutrient, pH, oxygen and temperature.

They require the elements present in their chemical composition. Nutrients must provide these elements in a metabolically accessible form.

## 2.0 Objectives

At the end of this unit, you should be able to:

- itemise nutrient requirements of microorganisms
- describe the nutritional types of microorganisms
- state the requirements for carbon, hydrogen, oxygen and electrons
- identify microbial growth factors
- describe nutrient uptake mechanisms in bacteria
- describe culture media
- explain the different phases of bacterial growth
- explain factors that influence bacterial growth.

## 3.0 Main Content

### 3.1 Common Nutrient Requirements

The nutrients may be in form of:

- Macronutrients or macroelements
- Micronutrients or trace elements

**Macronutrients**

These include calcium, oxygen, hydrogen, nitrogen, sulphur, phosphorus, potassium, Calcium, magnesium and iron (C, O, H, N, S, P, K, Ca, Mg, and Fe).

They constitute over 95% of cell dry weight and are needed in relatively large quantities.

C, O, H, N, S, and P are components of carbohydrates, lipids, proteins, and nucleic acids while the remaining four elements (K, Ca, Mg, F) exist in the cell as cations and play a variety of roles.

**Micronutrients**
These include manganese, zinc, cobalt, molybdenum, nickel and copper (Mn, Zn, Co, Mo, Ni, and Cu). They are used in very small amounts. In nature, they are ubiquitous and probably do not usually limit growth.

Requirements for Carbon, Hydrogen, Oxygen and Electrons

- All organisms require a source of carbon, hydrogen, oxygen, and electrons.
- Carbon is needed for the skeleton of all the organic molecules from which organisms are built.
- Hydrogen and oxygen are also important elements in organic molecules.
- The movement of electrons through the electron transport chain and during oxidation-reduction reactions it provides energy for cellular work.

## 3.2 Nutritional Types of Microorganisms

Microorganisms can be grouped into three sources based on their nutritional needs for growth.

Carbon sources:
- Autotrophs: use $CO_2$ as their primary source of carbon; they must obtain hydrogen and electrons from other sources.
- Heterotophs: use organic molecules as their source of carbon.

These molecules often supply hydrogen, oxygen, and electrons as well. Some heterotrophs also derive their energy from their organic carbon source.

Energy sources:
- Phototrophs: use light energy.
- Chemotrophs: obtain energy from oxidation of chemical compounds.

Electron sources:
- Lithotrophs: Electrons are extracted from reduced inorganic substances
- Organotrophs: Electrons are extracted from reduced organic comounds.

## 3.3 Growth Factors

These are organic factors that are essential cell components or precursors of such components but cannot be synthesised by the organism.

The three major classes are:

- Amino acids
- Purines and pyrimidines
- Vitamins

Vitamins are small organic molecules that usually are components of enzyme cofactors (riboflavin, folic acid etc).

Practical applications: microbes needing a growth factor can be used in bioassays that detect and quantify the growth factor; those that do not need a growth factor can sometimes be used to produce the growth factor in industrial settings.

## 3.4 Nutrient Uptake

Microorganisms make use of several different transport mechanisms:

- Facilitated diffusion
- Active transport
- Group translocation.

Although some nutrients can enter cells by passive diffusion, a membrane carrier protein is usually required.

Facilitated Diffusion - the transport protein simply carries a molecule across the membrane in the direction of decreasing concentration, and no metabolic energy is required.

Active transport systems use metabolic energy and membrane carrier proteins to concentrate substances actively by transporting them across a gradient. ATP is used as energy source by ABC transporters. Gradients of protons and potassium ions also drive solute uptake across membranes.

Bacteria also transport organic molecules while modifying them, a process known as group translocation e.g. many sugars are transported and phosphorylated simultaneously.

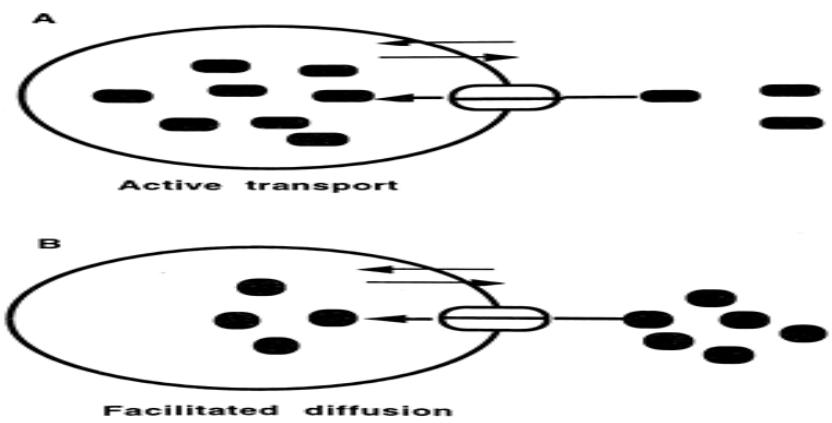


*Fig1: a. Active Transport b. Facillitated Diffusion* ***(Source:*** *Flylib.com****)***

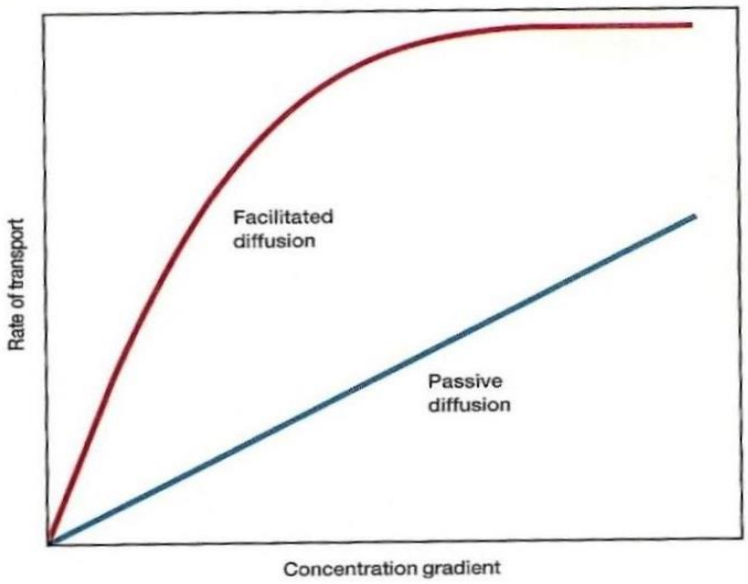


**Fig 2: Movement of Nutrients Across the Cell Membrane**
***Source:*** *Learning.Uonbi.Ac.Ke*

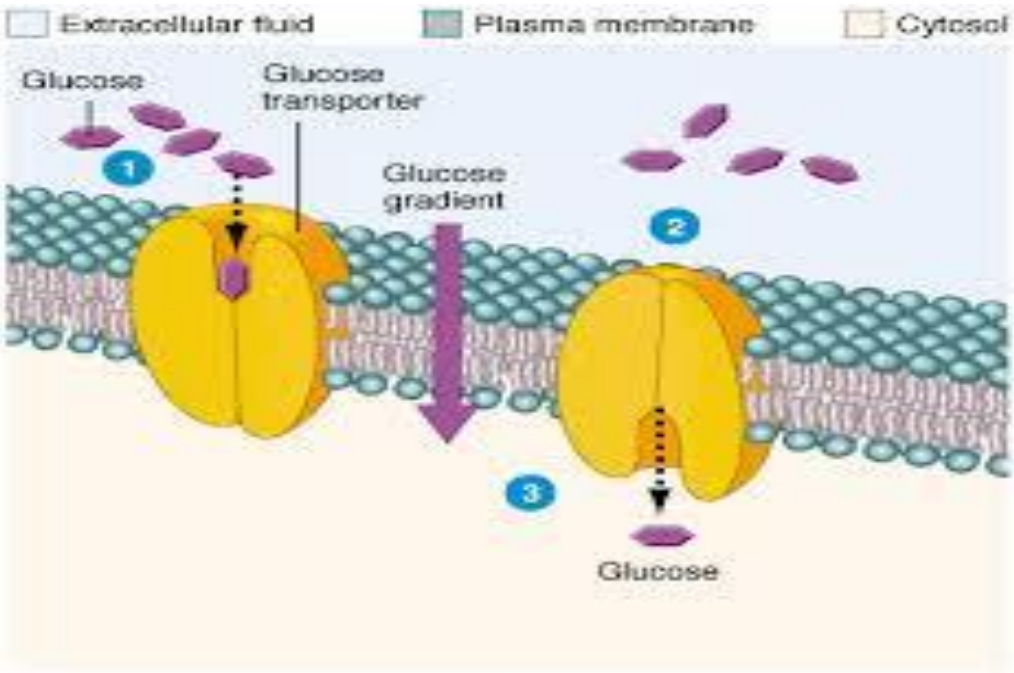


**Fig 3: Facilitated Diffusion: Carrier-Mediated Uptake of Glucose into the Cell**
**Source:** Classroom.Sdmesa.Edu

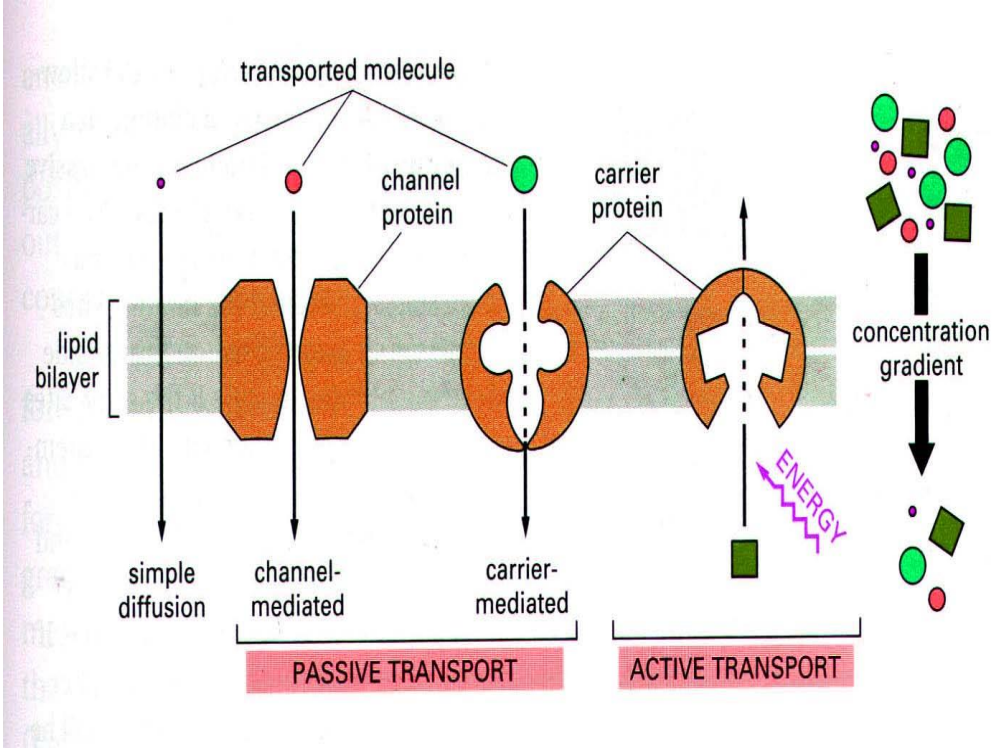


Fig.*4: Transportation of Nutrient Across the Cell Membrane*

**Source:** 12knights.Pbworks.Com

## 3.5 Culture Media

Culture media are solid or liquid preparation used to grow, transport, and store organisms. An effective medium must contain all the nutrients the microorganism requires for growth.

They are classified on the basis of several parameters:

- Chemical constituents from which they are made
- Physical nature
- Function.

### Types of Media

| Physical Nature | Chemical Composition | Functional Type |
|---|---|---|
| Liquid | Defined (synthetic) | Supportive (general purpose) |
| Semisolid | Complex | Enriched |
| Solid | | Selective |
| | | Differential |

Culture media can be constructed completely from chemically defined components (defined or synthetic media) or constituents like peptones and yeast extract whose precise composition is unknown (complex media).

Culture media can be solidified by the addition of agar, a complex polysaccharide from red algae.

### Classification

Enriched media are supportive media that contain additional nutrients needed by fastidious microbes.

Selective media contain components that select for the growth of some microbes.

Differential media contain certain components that allow microbes to be differentiated from each other, usually based on some metabolic capability.

TABLE 6.5 **Culture Media**

| Type | Purpose |
|---|---|
| Chemically defined | Growth of chemoautotrophs and photoautotrophs, and microbiological assays. |
| Complex | Growth of most chemoheterotrophic organisms. |
| Reducing | Growth of obligate anaerobes. |
| Selective | Suppression of unwanted microbes; encouraging desired microbes. |
| Differential | Differentiation of colonies of desired microbes from others. |
| Enrichment | Similar to selective media but designed to increase numbers of desired microbes to detectable levels. |



*Fig. 5*

Cultivation of Microorganisms

- This involves:
- Isolation
- Identification
- Preservation

## 3.6 Bacterial Growth

Growth is an orderly increase of all the components of an organism and not merely of some of its constituents. Growth occurs in various nutrient-containing preparations - culture media.

**The population of cells is referred to as a culture.**
Growth occurs first by increasing the amount of cellular organelles and then later through binary fission, in which a parent cell divides to form a progeny of two cells.

The time required for a single cell or population of cells to double is called the generation or doubling time. A population of bacterial cells goes through a number of phases from the time it is introduced into the medium until it ceases growth typified by the growth curve:

A "Typical" Bacterial Growth Curve

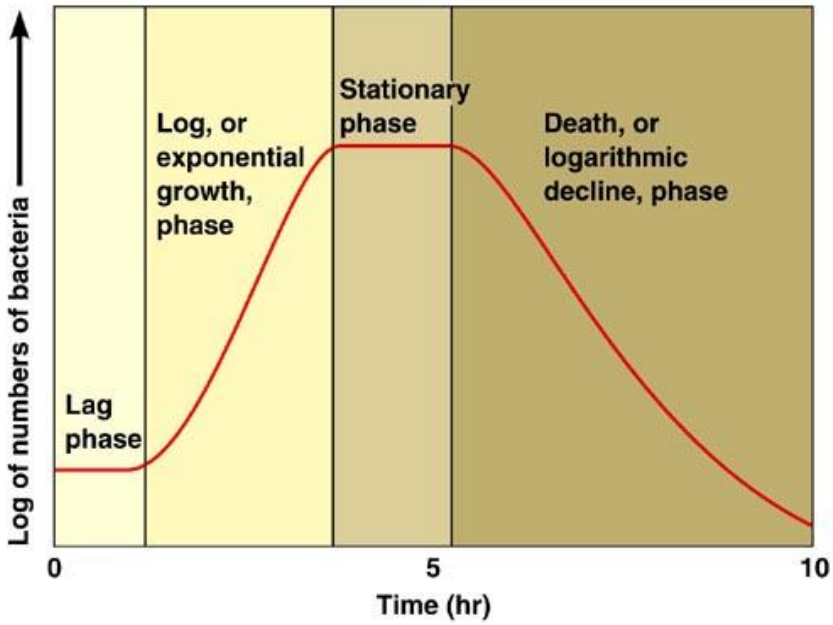


*Fig 6:*

Lag Phase -During this time, the organism adapts itself to the new environment with cell numbers remaining constant. There is considerable increase in RNA and total protein content of each cell but the DNA content remains approximately the same. The length of this phase usually depends on the physiological condition and size of the innoculum used.

Logarithmic or exponential phase -In this phase the organisms are growing at the maximum rate achievable in the medium employed, the cells dividing at minimum generation period with cell concentration increasing exponentially. The cells in this phase are at the peak of their metabolic activity hence they are most frequently used in experimental studies. The duration of this phase depends on whether or not the organisms in question are fast or slow growing.

Stationary Phase-After a phase of active growth, there follows a phase when once again there is little or no increase in the number of organisms so that the number of organisms remains constant. One explanation for this is probably that there is exhaustion of essential

nutrients and energy sources resulting from the activities of the exponential growth phase. Other reasons might be the accumulation of toxic metabolic wastes in the culture medium.

Death or Decline Phase -This is essentially the reverse of the exponential growth phase with the cells dying in a geometric progression fashion. The total cell counts may remain constant initially but the total number of viable cells continues to decline. This pattern is ascribable to the increase in toxic metabolites within the medium as well as the release of lytic enzymes by the dying cells.

The type of growth described above is known as batch culture. However, it is possible to use an open system in which there is a continuous supply of fresh nutrients into the culture medium and a continuous removal of grown bacteria by means of a constant level device. This type of continuous culture system is achieved by a chemostat or a turbidostat.

## 3.7 Environmental Factors Influencing Growth

- These factors include:
- pH: (negative logarithm of hydrogen ion concentration) - most bacteria grow best between pH 6 and 8. Some however are sensitive to acid but tolerant of alkali e.g. vibrio cholerae
- Temperature: each bacterium multiplies best within a restricted temperature range. Psychrophiles - grow below $20^0$C, usually quite well below $0^0$C. For example, soil and water bacteria. They cause spoilage of refrigerated and frozen food.
- ➢ Mesophiles: most cause disease in humans. T°: 30°-37°C.
- ➢ Thermophiles: are incapable of growth at the normal body temperature.
- ➢ They are not involved in infectious disease of humans. T° is 45 – 70°C.
- ➢ They are cause of spoilage in under-processed canned foods, since many form spores of exceptionally high heat-resistance.
- Osmotic Pressure- as a result of the presence of semi-permeable cytoplasmic membrane, bacterial resembles other cells in being subject to osmotic pressure. Sudden exposure of bacteria to solution of high salt concentration causes loss of water from the cells, and shrinkage of protoplast (plasmolysis). Plasmolysis prevents growth.
- Oxidation-Reduction (Redox) potential
- Carbon Dioxide
- Moisture and Dessication
- Light and other Radiations

Gaseous nutrients- it is necessary to provide oxygen for a strict aerobe and to remove it completely from the environment of strict anaerobe.

| Descriptive Term | Definition | Representative Microorganisms |
|---|---|---|
| **pH** | | |
| Acidophile | Growth optimum between pH 0 and 5.5 | |
| Neutrophile | Growth optimum between pH 5.5 and 8.0 | *Escherichia,* |
| Alkalophile | Growth optimum between pH 8.0 and 11.5 | |
| **Temperature** | | |
| Psychrophile | Grows well at 0°C and has an optimum growh temperature of 15°C or lower | *Bacillus psychrophilus* |
| Psychrotroph | Can grow at 0-7°C; has an optimum between 20 and 30°C and a maximum around 35°C | *Listeria monocytogenes*, *Pseudomonas fluorescens* |
| Mesophile | Has growth optimum around 20-45°C | *Escherichia coli, Neisseria gonorrhoeae, Trichomonas vaginalis* |
| Thermophile | Can grow at 55°C or higher; optimum often between 55 and 65°C | *Geobacillus stearothermophilus, Thermus aquaticus* |
| Hyperthermophile | Has an optimum between 80 and about 113°C | |
| **Oxygen Concentration** | | |
| Obligate aerobe | Completely dependent on atmospheric $O_2$ for growth | *Micrococcus luteus, Pseudomonas, Mycobacterium;* Most protists and fungi |
| Facultative anaerobe | Does not require $O_2$ for growth, but grows better in its presence | *Escherichia, Enterococcus* |
| Aerotolerant anaerobe | Grows equally well in presence or absence of $O_2$ | *Streptococcus pyogens* |
| Obligate anaerobe | Does not tolerate $O_2$ and dies in its presence | *Clostridium, Bacteroides* |
| Microaerophile | Requires $O_2$ levels below 2-10% for growth and is damaged by atmospheric $O_2$ levels (20%) | *Campylobacter, Treponema pallidum* |

## 4.0 Conclusion

In this unit, we have learnt about how bacteria grow and their nutrition. Also discussed were factors such as carbon dioxide, light, moisture and dedication and how they affect bacteria growth.

## 5.0 Summary

In this unit, you have learnt about the following:

- Common nutrients requirement
- Nutritional types of microorganisms
- Growth factors
- Nutrient uptake
- Culture media
- Bacterial growth
- Environmental factors influencing growth.

## 6.0 Self-Assessment Exercise

Activity: Discuss how environmental factors like carbondioxide, moisture and desiccation, light and other radiations affect the growth of bacterial.

Answer the following questions:

1. What are the nutrients requirements of microorganisms (LO1)
2. Explain the nutritional types of microorganisms (LO2)
3. Outline the requirements for carbon, hydrogen, oxygen and electrons (LO3)
4. What are microbial growth factors (Lo4)
5. Explain the following terms:
    a. Facilitated diffusion
    b. Active transport
    c. Group translocation (LO5)
6. Classify culture media and mentioning their uses (LO6)
7. Describe a "typical" bacterial growth curve (LO7)
8. The period between inoculation of bacteria in a culture medium and beginning of multiplication is known as:
    a. Lag phase
    b. Log phase
    c. Stationary phase
    d. Decline phase (LO7)

9. When a substance is added to a solid medium which inhibits the growth of unwanted bacteria but permits the growth of wanted bacteria, it is known as:

    a. Selective medium
    b. Enrichment medium
    c. Enriched medium
    d. Differential medium (LO6)

## 7.0 References/ Further Reading

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# Unit 2 Classification and Mode of Action of Antimicrobial

## 1.0 Introduction

A chemotherapeutic drug is a chemical compound that is used in the treatment of disease. The compound may come from natural sources or may have been synthesised by a chemist in the laboratory. An antibiotic is an antimicrobial agent that is derived from a microorganism while antimicrobial agent is a drug that acts primarily against infectious organisms.

Sir Flemming discovered Penicillin in 1928. Antibiotics were originally natural, produced by other organisms, but most are now semi-synthetic –modified from the original compounds (eg beta-lactams), a few are completely synthetic (eg quinolones, oxazolidinones, sulfanamides) while drugs like the aminoglycosides are still produced from living organisms.

## 2.0 Objectives

At the end of this unit, you should be able to:

- explain the classification of antimicrobials
- classify antibiotics according to site of action
- explain mode of action of each group with specific examples.

## 3.0 Main Content

### 3.1 Classification of Antimicrobials (Majorly Antibacterial)

- Bacteriostatic or bacteriocidal
- Site of action
- Chemical structure
- Range of activity.

### 3.2 Bacteriostatic

Inhibit growth of the microorganism at normal concentrations. Duration of therapy must be sufficient to allow cellular and humoral defense mechanisms to eradicate the bacteria. Final elimination is dependent on host immune system. Examples are: Tetracyclines, Erythromycin, Sulphonamides, and Chloramphenicol.

## 3.3 Bacteriocidal

Kill the microorganism. Bactericidal antibiotics should be used to treat infections of the endocardium or the meninges. Host defenses are relatively ineffective in these sites .Dangers imposed by such infections require prompt eradication of the organisms. E.g., Aminoglycosides, Fluoroquinolones, Penicillins, Cephalosporins.

## 3.4 Sites of Action

There are five major modes of action:

- interference with cell wall synthesis,
- inhibition of protein synthesis,
- interference with nucleic acid synthesis, and
- Inhibition of a metabolic pathway.
- Disruption of bacterial membrane structure.

Interference with Cell Wall Synthesis

- β-lactam agents inhibit synthesis of the bacterial cell wall by interfering with the enzymes (PBPs) required for the synthesis of the peptidoglycan layer. Eg: penicillins, cephalosporins, carbapenems, monobactams.
- Glycopeptide also interfere with cell wall synthesis, but by binding to the terminal D-alanine residues of the nascent peptidoglycan chain, thereby preventing the cross-linking steps required for stable cell wall synthesis. Eg vancomycin, teicoplanin.

β-lactams
The 1st antibiotic discovered was a β-lactam, i.e., penicillin in 1928 by Alexander Flemming

The work of Florey, Chain and associates in 1941 made possible the commercial production of penicillin G. All β-lactam antibiotics have a β-lactam nucleus in their molecular structure. We also have Penicillins and Derivatives such as cephalosporins, carbapenems, monobactams and β-lactam inhibitors.

The basic structure consists of a thiazolidine ring – the β-lactam ring – and a side chain. The β-lactam ring is essential for antibacterial activity. The side chain determines in large part the antibacterial spectrum and pharmacologic properties.

Examples of β-Lactams

a. Penicillins- penicillin G, penicillin V

Penicillinase – resistant penicillin

- Methicillin, nafcillin
- Isoxazolyl Penicillins - cloxacillin, Flucloxacillin, Oxacillin

Aminopenicillins -

Ampicillin, Amoxicillin, Bacampicillin,

Antipseudomonal (ureidopenicillins)-

Azlocillin, Carbenicillin, ticarcillin, Mezlocillin, Piperacillin

b. Carboxypeniciliins-

Carbenicillin, ticarcillin

c. Cephalosporins - discovered as naturally occurring substances from the mould Cephalosporium Cephalosporin C, obtained from the cultures of Cephalosporium acremonium and is the foundation on which current cephalosporin antimicrobials are constructed.

The β-lactam ring is fused to a six-membered dihydrothiazine ring (yielding the cephem nucleus). Contrast to penicillins in which the comparable unit is a five-membered thiazolidine ring.

Generations of cephalosporins- Based on spectrum of activity and timing of the agent's introduction.

1st generation: Relatively narrow spectrum of activity focused primarily on the gram-positive cocci. Eg - cephalothin , cephradine

2nd generation: Variable activity against gram-positive cocci but has increased activity against gram-negative bacteria. Eg - cefuroxime, cefoxitin

3rd generation: Very marked activity against the gram-negative bacteria; some of them have limited activity against gram-positive cocci, particularly MSSA. E.g. - Cefotaxime (claforan), Ceftriaxone (rocephin), Ceftazidime (fortum), Cefoperazone.

4th generation. Good true broad-spectrum activity against both Gram-negatives and Gram-positives. Eg Cefepime

5th generation - MRSA-active cephalosporins and currently includes ceftaroline and ceftobiprole.

Cephamycins- Closely related to cephalosporins. They contain oxygen in place of sulfur in the dihydrothiazine ring, rendering them more stable to beta-lactamase hydrolysis. The cephamycins are noted for their additional activity against gram-negative anaerobic bacteria, such as Bacteroides spp. They are grouped together as Second generation cephalosporins. E.g. cefoxitin, cefotetan, cefmetazole.

Other β-lactams;

- Monobactams : narrow-spectrum antibiotics .Active only against aerobic, gram-negative bacteria.eg Aztreonam
- Carbapenems: They are derivatives of thienamycin, a compound produced by streptomyces cattleya. They diffuse easily in bacteria and are considered as broad-spectrum antibiotics active against virtually all groups of organisms with few exceptions (such as Stenotrophomonas maltophilia). Eg Meropenem , Imipenem and Ertapenem

β-lactamase Inhibitors (suicide inhibitors)- Do not contain the β-lactam ring

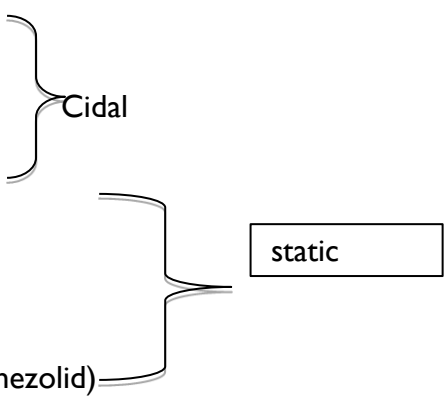
- Clavulanate, Sulbactamt, Azobactam: They can be combined with other β-lactams eg amoxycillin to enhance antimicrobial spectrum. They do not affect the pharmacokinetics nor does it increase side -effects. It Increases resistance to β-lactamases. Possess negligible antimicrobial activity. May be reversible or irreversible. All used in clinical practice are irreversible.

➢ Amoxycillin-clavulanate (Augmentin)

- Ampicillin-sulbactam (Unasyn)
- Ticarcillin-clavulanate (Timentin)
- Piperacillin-tazobactam (Zosyn)

Other Inhibitors of cell wall synthesis asides β – lactams.

- Glycopeptides - Vancomycin, Teicoplanin, Ramoplanin, Decaplanin
- Bacitracin
- Cycloserine
- Fosfomycin
- Inhibition of Protein Synthesis.
- Antibiotic Classes
    - Aminoglycosides
    - Streptogramin
    - Glycylcyclines
    - Tetracyclines
    - Chloramphenicol
    - Macrolides
    - Lincosamides
    - Fusidic acid
    - Oxazolidones (Linezolid)




Antimicrobials that Attack the 30s Ribosomal Subunit Blocking Protein

Synthesis Aminoglycosides - The antibiotics inhibit bacterial protein synthesis by irreversibly binding to 30S ribosomal proteins.

Originally they were isolated from Streptomyces species. Gentamicin was isolated from Micromonospora species. Amikacin is a synthetic derivative of kanamycin. Broad spectrum: Act in synergy with other agents e.g. Streptomycin, neomycin, kanamycin, tobramycin.

Tetracyclines: Tetracycline, Oxytetracycline, Doxycycline and Minocycline, they are oral absorptions – poor with food, milk, orange juice, antacids iron containing tonics. Mode of action is by reversible binding to the 30S ribosome and inhibition of binding of aminoacyl-t-RNA to the acceptor site on the 70S ribosome.

Spectrum of Activity – Broad spectrum; Useful against intracellular bacteria. Resistance is Common with Adverse effects including Destruction of normal intestinal flora resulting in increased secondary infections and staining of the structure of bone and teeth.

Glycylcycline which is Tigecycline.: Is a synthetic analogue of Tetracycline. The broad spectrum which is useful against strains resistant to tetracyclines and other antibiotics has Clinical use: skin and soft tissue, intra-abdominal infections.

Spectinomycin- reversibly interferes with m-RNA interaction with the 30S ribosome. It is structurally similar to aminoglycosides but does not cause misreading of mRNA . Used in the treatment of penicillin-resistant Neisseria gonorrhoeae.

Oxazolidinones - Linezolid: Attach to 30s ribosomes. Affect translation by inhibiting the formation of N-formylmethionyl-tRNA activity mainly against gram positive organisms.

Antimicrobials that Attack the 50s ribosomal subunit- blocking protein

**Synthesis**
Chloramphenicol, lincomycin, clindamycin - bind to the 50S ribosome and inhibit peptidyl transferase activity. Chloramphenicol is broad spectrum while Lincomycin and clindamycin are narrow spectrum. Resistance is however common Chloramphenicol can be toxic (bone marrow suppression) but it is used in the treatment of bacterial meningitis. Lincomycin and clindamycin predispose to Pseudomembranous colitis.

Macrolides– Erythromycin, Azithromycin, roxythromycin, clarithromycin. Inhibit translocation in protein synthesis. Good coverage against Gram-positive bacteria, Mycoplasma, Legionella.

Streptogramins- (Bacteriocidal) Eg: Quinupristin/dalfopristin. Synergistic activity when used together causing irreversible binding to different sites of the 50S ribosome. Good against Gram positive organisms with little resistance developed.

**Inhibitors of Nucleic Acid Synthesis**
The inhibitors of the DNA replication Quinolones and fluoroquinolones are. family of synthetic antimicrobial agents is the first quinolone, nalidixic acid and was identified among by-products of chloroquine synthesis in 1962. NA has bactericidal activity against Gram-negatives.

2nd generation quinolones has a fluoride atom at position 6 of quinolone molecule with enhanced biological activity. Fluoroquinolones discovered in the 1980s e.g. ciprofloxacin, ofloxacin, perfloxacin, and norfloxacin.

3rd generation – (fluoro) quinolones e.g. levofloxacin with activity against both Gram-positives and Gram-negatives

The quinolones selectively interfere with bacterial DNA replication by inhibiting two enzymes involved in DNA synthesis: the type II topoisomerase known as DNA gyrase, and DNA topoisomerase IV. They generally have broad spectrum activity.

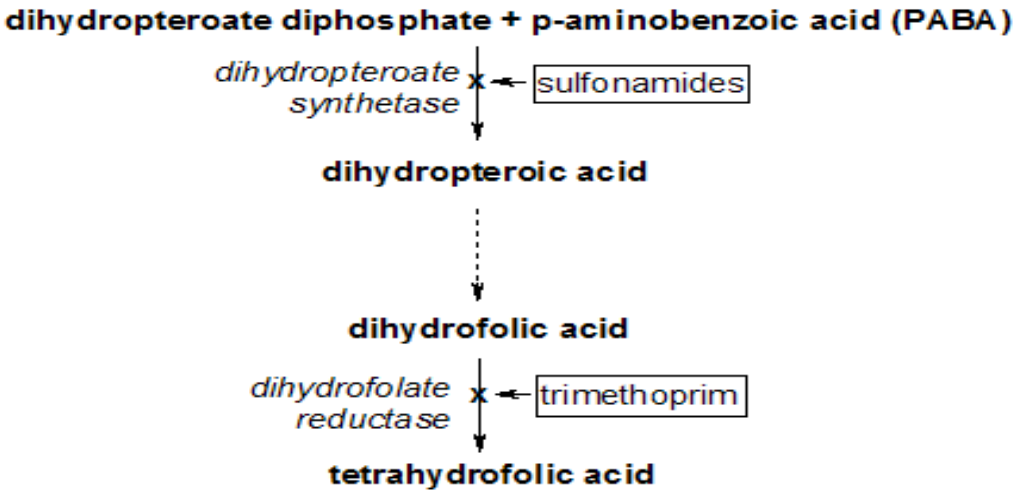
Wide spectrum of action but is used most commonly in the treatment of tuberculosis. Since resistance is common, rifampin is usually used in combination therapy.

Nitroimidazoles -Interact with DNA leading to breaks in the DNA – Metronidazole, Tinidazole. Active against anaerobes and some protozoa

Inhibition of a Metabolic Pathway.

**Inhibitors of Folic Acid Synthesis**
The selectivity of these antimicrobials is a consequence of the fact that bacteria cannot use pre-formed folic acid and must synthesise their folic acid. In contrast, mammalian cells use folic acid obtained from food.

Trimethoprim- Available since 1962. The last truly new antibacterial agent introduced into clinical practice. All late developed agents are variations of older antibiotics. It is completely synthetic.




Trimethoprim, methotrexate, pyrimethamine (bacteriostatic) - bind to dihydrofolate reductase and inhibit formation of tetrahydrofolic acid. Broad Spectrum and used primarily in urinary tract infections and in Nocardia infections. Resistance however is Common.

Sulfonamides, Sulfones (bacteriostatic) - analogues of para-aminobenzoic acid and competitively inhibit formation of dihydropteric acid.

Combination therapy – Trimethoprim used in combination with the sulfonamides. This combination blocks two distinct steps in folic acid metabolism and prevents the emergence of resistant strains Inhibition of Cell Membrane Function Antibacterial - Polymyxins; Very toxic (Nephrotoxic), used mainly topically and also the gram negative organisms, except Proteus cells. Polymyxin E– only one used parenterally (colistin). Polymyxin B- topical on skin binds to the lipid A portion of lipopolysaccharide and also to phospholipids. However, it binds preferentially to lipid A. This disrupts the outer membrane of Gram negative bacteria. Since the cell membrane is not exposed in Gram positive bacteria polymyxin has little activity against them. It is toxic to human cells, since it can also lyse eukaryotic membranes; hence has limited clinical use.

Antifungal Drugs- Polyenes –Nystatin and Amphotericin B . Bind to fungal ergosterol. It crosses reacts with human cholesterol.

Antibacterial – The cyclic lipopeptide, daptomycin inserts its lipid tail into the bacterial cell.

## 4.0 Conclusion

In this unit, we have learnt about different classes of antimicrobial and their mode of actions.

## 5.0 Summary

In this unit, we have learnt about the following:

- Classification of antimicrobials (majorly antibacterial);
- Bacteriostatic

- Bacteriocidal
- Sites of action.

## 6.0 Self Assessment

Activity:

What are the groups of Antibiotics commonly used in your hospital and what inform the choice of these groups of drugs?

Answer the following questions:

1. Describe the mode of action of penicillins (LO1)
2. List the generations of cephalosporin and two examples each (LO1)
3. Explain how antimicrobials inhibit protein synthesis (LO2)
4. List antimicrobials that affect the nucleic acid of organisms (LO2)
5. Write short note on the following

a. Bacteriostatic
b. Bacteriocidal (LO1).

6. Describe antimicrobials that attack the 305 ribosomal subunit blocking protein synthesis (LO3)

## 7.0 References/ Further Reading

Atlas, R.M. (1995). *Microorganisms in Our World*. Mosby Year Book.Inc.

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# Unit 3 Sterilisation and Disinfection

## 1.0 Introduction

In healthcare settings, various surgical and medical procedures are usually performed. These procedures involve contact by medical devices or surgical instruments with patients' sterile tissues or mucous membrane. A major risk of all such procedures is the introduction of pathogens that can lead to infection.

Failure to properly disinfect or sterilise equipment carries not only risk associated with breach of host barriers but also risk for person-to-person transmission (e.g. of hepatitis B virus) and transmission of environmental pathogens (e.g. Pseudomonas aeruginosa).

## 2.0 Objectives

At the end of the unit, you should be able to:

- state the basis of infection control practices.
- list the principles of infection control
- differentiate between sterilisation and disinfection
- itemise various methods available for sterilisation and disinfection, including the newer disinfectants
- explain a disinfection policy.

## 3.0 Main Content

### 3.1 Basis of Infection Control Practices

Involves use of practices and procedures that prevent or reduce the likelihood of infections being transmitted from a source e.g., person, contaminated body fluids, equipment, and environment to a susceptible individual.

### 3.2 Principles of Infection Control

These include:

1. Handwashing
2. Protective Clothing
3. Cleaning Disinfection and Sterilisation
4. Management of Linen
5. Management of Waste
6. Management of Blood spillage
7. Management of Inoculation and Contamination incidents
8. Specimen handling and transportation.

## 3.3 Definition of Terms

Cleaning- The physical removal of organic material or soil from objects. It involves use of water with or without detergents. It removes; not to kill microbes.

Sterilisation - The total elimination of all forms of microbial life including spores

Disinfection - These is the elimination of vegetative organisms without elimination of spores.

## 3.4 Rationale for Choice of Procedure

This entails categorizing medical devices, equipment and surgical materials on the basis of risk of causing infection, into:

- Critical items
- Semi-critical items
- Non-critical items.

**Critical Items**
These are instruments or objects that will be introduced directly into the blood stream or into other normally sterile areas. These include surgical instruments, implants, blood compartment of a haemodialyzer, cardiac catheters. The minimum standard required is sterilisation.

**Semi-Critical Items**
These items come in contact with intact mucosal surfaces but do not ordinarily penetrate body surfaces. They have intermediate risk of causing infection. These include non-invasive flexible and rigid endoscopes, endotracheal tubes, cystoscopes, anaesthesia breathing circuits.

Sterilisation is preferred but is not absolutely essential. A high-level disinfection procedure that can be expected to destroy vegetative microbes, most fungal spores, tubercle bacilli and small non-lipid viruses can be recommended.

**Non-Critical Items**
These items do not ordinarily touch the patient or touch only intact skin and have a low risk of transmitting infection. These items include crutches, blood pressure cuffs, stethoscopes, tourniquets etc. Cleaning with water and detergent may be adequate though a low-level disinfectant may be preferred in all cases.

## 3.5 Sterilisation

There are various methods available for sterilisation. For example, use of:

- Moist heat under pressure(autoclaving)
- Dry heat
- Ethylene oxide gas
- Vapor phase Hydrogen peroxide

- Ionizing radiation.

Moist Heat under Pressure (Autoclaving)

- This involves autoclaving at 121°C for 15mins /flash sterilisation 270°C for 3mins (not for implants).
- It is very reliable and efficient.
- It can be used to sterilise dressings, instruments, glasswares.
- It is not suitable for powders, some plastics, anhydrous oils (i.e., heat & moisture sensitive)

***Fig: 1: An Autoclave***

**Dry Heat**

- Sterilisation using dry heat at 160°C for 2hrs. Hot air oven is used.
- This method is used for heat stable materials, glassware, oils, powders.

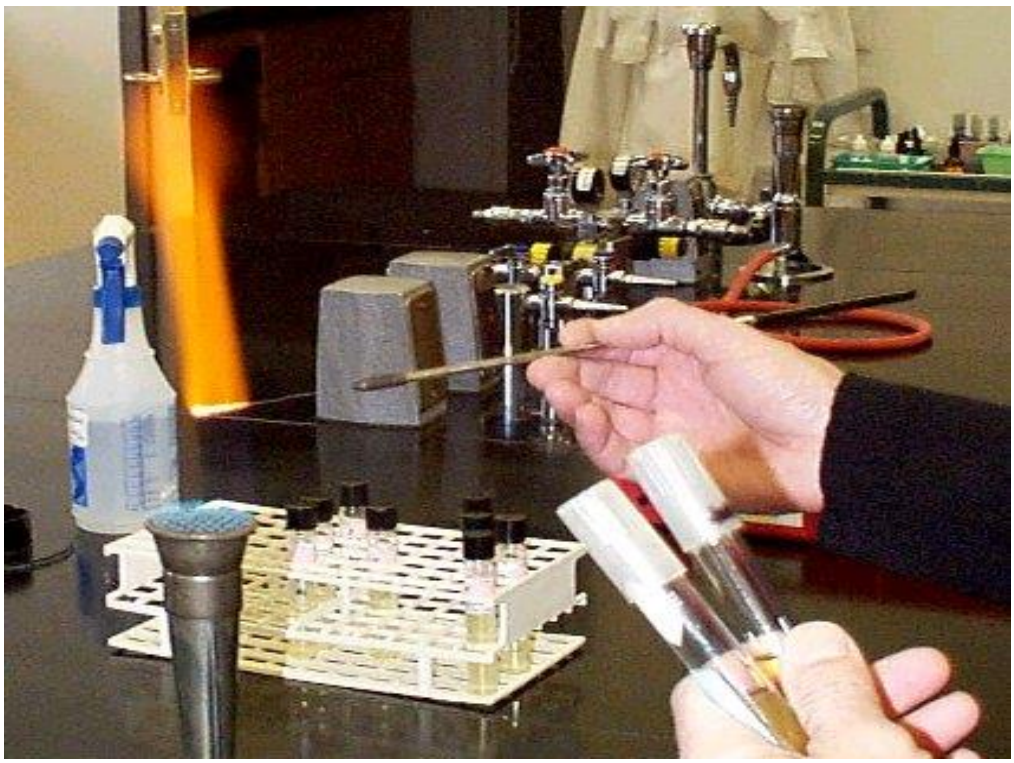

**Fig: 2: Flaming**


Ethylene Oxide Gas

- This is effective at low temperature.
- It has good penetrating power, and compatible with most medical materials.
- However, it is expensive, toxic and inflammable.

Vapor Phase Hydrogen Peroxide

- Sterilisation occurs at low temp. It is safe, no toxic residuals; simple to operate, install and monitor.
- Sterilisation takes place in small chamber. It requires synthetic packaging.
- Devices with long or narrow lumens cannot be processed.

**Ionising Radiation**

This sterilisation method is used for packaging materials, mainly industrial. For example, use of cobalt 60 gamma rays or electron accelerators.

Central Sterile Services Department (CSSD)

- Cleaning, Disinfection and Sterilisation of patients' supplies should be performed in the CSSD.
- It should be divided into several areas separated by physical barriers.
- Temperature should be between 18°C – 22°C.
- Relative humidity should be between 35% -70%.
- Airflow should be directed from clean to relatively soiled areas.

Rules of Sterilisation

- All items should be thoroughly cleaned before Sterilisation
- Presoaking in disinfectants is ineffective and should be discouraged
- Where possible precleaning should be automated
- All items should be double wrapped
- Wrapping should be compatible with the sterilisation process, inexpensive, impervious to bacteria (140-thread-count muslin, kraft paper etc.), durable, flexible, free of pinholes.

**Monitoring the Sterilisation Process**

Physical monitoring: Monitoring temperature, pressure, time.

Chemical monitoring: Involving colour or physical change indicators that monitor exposure to sterilizing agents/conditions.

Biological monitoring: This is the most important monitoring method. It is done weekly.

Bacillus stearothermophilus is the biological indicator used for sterilisers and Bacillus subtilis var niger or var.globigii for Ethylene oxide sterilisers.

## 3.6 Disinfection

Methods of Disinfection

Broadly divided into:

- Boiling - Occurs at 100°C. It is not sporicidal.
- Chemical Disinfection- Disinfectants are of different types. Their actions are dependent on many factors.

Classification of Chemical Disinfectants based on their microbicidal activity into:

1. High level disinfectants

2. Intermediate level disinfectants
3. Low level disinfectants.

Low Level Disinfectants: They can kill only vegetative bacteria and enveloped viruses but not the tubercle bacilli, spores or small and non-lipid (non-enveloped) viruses; though they may kill fungi after prolonged contact.

Intermediate Level Disinfectants: They kill vegetative bacteria, tubercle bacilli fungi and enveloped viruses. They have no effect on spores and non-enveloped (non-lipid) viruses at normal contact times. They may exhibit limited virucidal (against non-enveloped) activity on prolonged contact. Examples include Chlorine compounds, Alcohols.

High Level Disinfectants: They kill everything except spores. At extended contact times they are capable of actual sterilisation. Examples include 2% gluteraldehyde, Heat, Chlorine dioxide, Peracetic acid.

**Factors Affecting Disinfectants**

These include:

- Type (Chemical component)
- Concentration
- pH of the medium
- Temperature
- Volume
- Contact time
- Length of storage
- Nature and amount of contamination
- Presence of inactivating substances
- Surface to be disinfected
- Prior cleaning.

It is Impotrant to always use Disinfectants according to the Manufacturer's Instruction

Types of Disinfectants

- Aldehydes – e.g., Gluteraldehyde/formaldehyde
- Halogens – e.g., Hypochlorite/chlorine
- Alcohols – e.g., Isopropyl/ethyl/methyl
- Chlorhexidine – e.g., Hibiscrub (chl + 4%detergent), Hibisol (chl + alcohol + glycerine), Chl. Ointment
- Iodophors/iodine – e.g., Povidone iodine
- Phenolics – e.g., Phenol, Chloroxynelol, Hexachlorophen. They are environmental & laboratory disinfectants
- Quanternary Ammonium Compounds- e.g., Cetrimide.

Newer Disinfectants
Peracetic Acid – NuCidex

They are:

- Strong oxidising agent
- Rapid bactericidal agent
- High level disinfectant.

Peroxygen Based Compounds-Virkon

They are:

- Broad spectrum
- Good environmental disinfectant (may damage equipments)
- For semi-critical items

**Superoxidised Water**

They:

- Are environmentally friendly
- Have broad spectrum of action
- May be useful for semi-critical items

**Rules for Use of Disinfectants**

- Follow manufacturers' instructions
- Check Expiry date of solution
- Ensure optimum dilution
- Always wash and clean articles before disinfection
- Do not refill disinfectant containers between each use –
- Topping up is not allowed
- Disinfectants should be supplied ready for use from the pharmacy
- Return empties to pharmacy – do not discard or use for other purposes
- Do not use to sterilise instruments or equipment (unless specified in the disinfection policy)
- Open containers are a serious NO! NO! in any hospital environment
- Where disinfectants are indicated for use on surfaces, WIPE – DO NOT BATHE

**Disinfection Policy**

- List purposes for which disinfectants are used
- Identify unnecessary or dangerous practices
- Select effective disinfectants for remaining indications
- Ensure optimum dilution
- Arrange distribution and collection of empties/expired
- Ensure proper labeling
- Must be written and prominently displayed
- Must be reviewed every two years.

**The Role of the Pharmacy**

- Ensure cleanliness of containers
- Ensure proper dilution
- Ensure correct labeling
- Ensure proper distribution and collection

Some Myths about Disinfection in Operating Theatres

- Transfer areas where patients are transferred from ward trolleys to clean OR trolleys
- Routine culturing of OR personnel
- Routine culturing of the environment
- Ultraviolet rays for disinfecting theatres
- Disinfecting theatre floors
- Patient antiseptic baths or showers before surgery.

## 4.0 Conclusion

Discussed in this unit, were the sterilisation and disinfection are, why they need to be carried out and how they can be performed.

## 5.0 Summary

In this unit, you have learnt about the following:

- Basis of Infection Control Practices
- Principles of Infection Control
- Definition of Terms
- Rationale for Choice of Procedure
- Sterilisation
- Disinfection.

## 6.0 Self-Assessment Exercise

Activity: List the disinfectants you usually used and write out their solutions.

Answer the following questions:

1. What are the bases of infection control practices (LO1)?
2. List the principles of infection control (LO2)
3. Differentiate between sterilisation and disinfection (LO3)
4. Enumerate the various methods for sterilisation and disinfection (LO4)
5. Outline the newer disinfectants (LO4)

6. Explain a disinfection policy (LO5
7. The process of total elimination of all forms of microbial life, including spores is:
    - Sterilisation
    - Disinfection
    - Antisepsis
    - Cleaning
8. Heating in a hot air oven at $160^0$C for one hour is used for sterilisation of:
    - Glass syringes
    - Oils and jellies
    - Powders
    - All of the above.

## 7.0 References/ Further Reading

Atlas, R.M. (1995). *Microorganisms in Our World*. Mosby Year Book.Inc.

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