**COURSE CODE:** *MCB 308*
**COURSE TITLE:** *Pharmaceutical Microbiology*
**NUMBER OF UNITS**: *3 Units*
**COURSE DURATION:** *Three hours per week*

## COURSE DETAILS

**Course Coordinator: PROF. I. AKPAN**
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**Other lecturers: DR. S.O.KAREEM and DR. M.T. OBUOTOR**

## COURSE CONTENT

The chemistry of synthetic chemotherapeutic agents and antibiotics; production and synthesis of antibiotics and other antimicrobial agents, relationship of antimicrobial agents to different groups of microorganisms; the mode of action and assay of antimicrobial agents (concepts of antibiotic sensitivity and resistance as related to microbial physiology), quality control of pharmaceutical products; concepts of growth and death in microorganisms: history and applications antibiotic, antibiotic resistance mechanisms; discovery and approval of new antibiotics; sources of antibiotics role in nature).

## COURSE REQUIREMENTS

This is a compulsory course for 300 level students in the department of Microbiology. In view of this, students are expected to participate in all the course activities and have minimum of 75% attendance to be able to write the final examination.

## READING LIST

Kuntz, I.D. (1992) Structure-based strategies for drug design and discovery. *Science*, **257**, 1079–1082.
Patick, A.K. & Potts, K.E. (1998) Protease inhibitors as antiviral agents. *Clin Microbiol Rev*, **11**, 614–627.
Power, E.G.M. & Russell, A.D. (1998) Design of antimicrobial chemotherapeutic agents. In: Smith, H.J., ed. *Introduction to Principles of Drug Design*, 3rd edn. Wright, Bristol.
Reeves, D.S. & Howard, A.J. (1991) New macrolides— the respiratory antibiotics for the 1990s. *J Hosp Infect*, **19** (Suppl. A).

Russell, A.D. & Chopra, I. (1996) *Understanding Antibacteral Action and Resistance*, 2nd edn. Ellis Horwood, Chichester.
Sammes, P.G. (1997–1982) *Topics in Antibiotic Chemistry*, vols 1–5. Ellis Horwood, Chichester. Shanson, D.C. (1999) *Microbiology in Clinical Practice*, 3r edn. Wright, London

# LECTURE NOTES

**MCB 308 PHARMACEUTICAL MICROBIOLOGY 3UNITS**

**LECTURER : PROF. INYANG AKPAN**

**The chemistry of synthetic chemotherapeutic agents.**

**Definition**

**Chemotherapy**: The therapeutic concept developed by **Paul Ehrlich** (1854–1915) whereby a specific drug
or chemical is invariably employed to treat an ensuing infectious disease or cancer ; ideally, the chemical must destroy the **pathogens** completely without harming the host.

**Chemotherapeutic Agents.** Compounds used in the treatment of disease that destroy pathogens or inhibit their growth at concentrations low enough to avoid doing undesirable damage to the host.

**Antibiotics:** An antibiotic was originally defined as a substance,produced by one microorganism, which inhibited the growth of other microorganisms. The advent of synthetic methods has, however, resulted in a modification of this definition and an antibiotic now refers to a substance produced by a microorganism,
or to a similar substance (produced wholly or partly by chemical synthesis), which in low concentrations inhibits the growth of other microorganisms. Chloramphenicol was an early example.

**Bacteriocins** although produced by microorganisms are also not included in this definition because they are not only larger in molecular size than the usual antibiotics, but they are mainly protein in nature; furthermore they affect mainly organisms related to the producing organism. In comparison with bacteriocins, conventional antibiotics however are for more diverse in their chemical nature and attack organisms distantly related to themselves. Most importantly, while the information specifying the formation of 'regular' antibiotics is carried on several genes, that needed for bacteriocins being single proteins need single genes. Antimicrobial agents such as sulphonamides and the 4-quinolones ,produced solely by synthetic means, are often referred to as antibiotics

**Uses of Antibiotics:**

- Employed extensively to treat infectious, diseases in humans, animals, and plants.
- Employed in animal husbandry as *'feed additive'* to cause enhancement in the fattening of food animals.
- In food handling and processing industries to critically minimise inevitable spoilage of fish, vegetables, and poultry products.
- As useful and indispensable tools for the elaborated study of **biochemical cellular mechanisms in** scientific researches

**MILESTONE IN ANTIBIOTICS DISCOVERY.**

Since the discovery of **penicillin** many more antibiotics came into being as stated under :

Waksman (1944) : **Streptomycin** — [*Streptomyces griseus*] — a soil microbe ;
— (1945) : **Bacitracin** — [*Bacillus subtilis*] ;
— (1947) : **Chloramphenicol** (Chloromycetin) — [*Streptomyces venezuelae*] ;
— (1947) : **Polymixin** — [*Bacillus polymixa*] — and various designated polymixins A, B, C, D, and E
— (1948) : **Chlorotetracycline** — [*Streptomyces aureofaciens*] — a broad-spectrum antibiotic.
— (1948) : **Neomycin** — [a species of *Streptomyces*] — isolated from soil
— (1950) : **Oxytetracycline** — [a strain of *Streptomyces*].
— (1952) : **Erythromycin** — [*Streptomyces erythreus*].

Thousands of antibiotics are known; and every year dozens are discovered. However, only a small proportion of known antibiotics is used clinically, because the rest are too toxic.

**Classification and Nomenclature of Antibiotics**

Several methods of antibiotic classification have been adopted by various authors. The mode of action has been used, e.g. whether they act on the cell wall, or are protein inhibitors, etc. Several mechanisms of action may operate simultaneously making such a method of classification difficult to sustain. In some cases they have been classified on the basis of the producing organisms, but the same organism may produce several antibiotics, e.g. the production of penicillin N and cephalosporin by a *Streptomyces* sp. The same antibiotics may also be produced by different organisms. Antibiotics have been classified by routes of biosynthesis; however, several different biosynthetic routes often have large areas of similarity. The spectra of organisms attacked have also been used, e.g. those affecting bacteria, fungi, protozoa, etc. Some antibiotics belonging to a well known group e.g. aminoglycosides may have a different spectrum from the others. The classification to be adopted here therefore is based on the chemical structure of the antibiotics.

**CATEGORIES OF ANTIBIOTICS**

| S.No | Class | Designated Antibiotics |
|---|---|---|
| I | **Aminoglycosides** | Tobramycin Amikasin ; Gentamycin ; Kanamycin ; Neomycin ; Netilmicin, Streptomycin, |
| II | **Ansamycins** | Maytansine ; and Rifampicin ; |

| | | |
|---|---|---|
| III | **Beta-lactam antibiotics** | Thienamycin Amoxycillin;Ampicilinl Cephalosporin Clavulanic acid, Cloxacillin Nocardicins Penicillins; |
| IV | **Cyclic polypeptides** | Gramicidin ; and Polymixins A, B, C, D and E ; |
| V | **Fluoroquinolones** | Ciprofloxacin ; Enoxacin ; Norfloxacin ; Ofloxacin. |
| VI | **Macrolides** | Azithromycin ; Bacitracin ; Clarithromycin, Erythromycin ; |
| VII | **Polyenes** | Amphotericin B ; Griseofulvin ; and Nystatin ; |
| VIII | **Tetracyclines** | Aureomycin ; Doxycycline ; Minocycline ; Oxytetracycline ; Tetracycline ; |
| IX | **Miscellaneous** | Adriamycin,Chloramphenicol,(Chloromycetin) ; Clindamycin ;Cycloserine ; and Mitomycins. |

Source: [Kar, Ashutosh : **Pharmacognosy and Pharmacobiotechnology,** New Age International (P)Ltd., Publishers, New Delhi, 2003].

**NOTE**:
The nomenclature of antibiotics is also highly confusing as the same antibiotic may have as many as 13 different trade names depending on the manufacturers. Antibiotics are therefore identified by at least three names: the chemical name, which prove long and is rarely used except in scientific or medical literature; the second is the group, generic, or common name, usually a shorter from of the chemical name or the one given by the discoverer; the third is the trade or brand name given by the manufacturer to distinguish it from the product of other companies

## SYNTHESIS OF ANTIBIOTICS AND OTHER ANTIMICROBIAL AGENTS.

**Sources**
There are **three** major sources from which antibiotics are obtained.

**A Microorganisms.** Bacitracin and polymyxin are obtained from some *Bacillus* species; streptomycin, tetracyclines, etc. from *Streptomyces* species; gentamicin from *Micromonospora purpurea*; griseofulvin and some penicillins and cephalosporins from certain genera (*Penicillium, Acremonium*) of the family Aspergillaceae; and monobactams from *Pseudomonas acidophila* and *Gluconobacter* species. Most antibiotics in current use have been produced from *Streptomyces* spp.

**B Synthesis.** Chloramphenicol is now usually produced by a synthetic process.

**C Semisynthesis.** Part of the molecule is produced by a fermentation process using the **ap**propriate microorganism and the product is then further modified by a chemical process. Many penicillins and cephalosporins are produced in this way .In addition, it has been suggested that :
(a) some bacteriophages might have an important role to play in the chemotherapy of bacterial infections,

(b) plant products might prove to be a potentially fruitful source of new antimicrobial agents.

**2. b-Lactam antibiotics**

The Beta-lactam antibiotics are so-called because they have in their structure the four membered lactam ring as shown below:

There are several different types of b-lactam antibiotics that are valuable, or potentially important antibacterial compounds. The Beta-lactam antibiotics include the well-established and clinically important penicillins and cephalosphorins as well as some relatively newer members: cephamycins, nocardicins, thienamycins, and clavulanic acid. Except in the case of nocardicins these antibiotics are derivatives of bicyclic ring systems in which the lactam ring is fused through a nitrogen atom and a carbon atom to ring compound. This ring compound is five-membered in penicillins (thiazolidine), thienamycins (pyrroline) andclavulanic acid (oxazolidine); it is six-membered (dihydrothiazolidine) incephalosporins and cephamycins The Beta-lactam antibiotics inhibit the formation of the structure-conferring petidoglycan of the bacterial cell wall. These will be considered briefly.

**2.1 Penicillins**

The penicillins may be considered as being of the following types.

**1 Naturally** occurring.e.g, those produced by fermentation of moulds such as *Penicillium notatum* and *P. chrysogenum*. The most important examples are benzylpenicillin (penicillin G) and phenoxymethylpenicillin (penicillin V).

**2 Semisynthetic**. In 1959, scientists at Beecham Research Laboratories succeeded in isolating the penicillin 'nucleus', 6-aminopenicillanic acid. During the commercial production of benzylpenicillin, phenylacetic (phenylethanoic) acid (C6H5.CH2.COOH) is added to the medium in which the *Penicillium* mould is growing (see Chapter 22). This substance is a precursor of the side-chain in

benzylpenicillin. Growth of the organism in the absence of phenylacetic acid led to the isolation of 6-APA; this has a different *R*F value from benzylpenicillin, which allowed it to be detected chromatographically. A second method of producing 6-APA came with the discovery that certain microorganisms produce enzymes, penicillin amidases (acylases), which catalyse the removal of the side-chain from benzylpenicillin Acylation of 6-APA with appropriate substances results in new penicillins being produced that differ only in the nature of the side-chain Some of these penicillins have considerable activity against Gram-negative as well as Grampositive bacteria, and are thus broad-spectrumBenzylpenicillin is rapidly absorbed and rapidly excreted. However, certain sparingly soluble salts of benzylpenicillin (benzathine, benethamine and procaine) slowly release penicillin into the circulation over a period of time, thus giving a continuous high concentration in the blood. Pro-drugs (e.g. carbenicillin esters, ampicillin ester ;are hydrolysed by enzyme action after absorption from the gut mucosa to produce high blood levels of the active antibiotic, carbenicillin and ampicillin, respectively. Several bacteria produce an enzyme, b_lactamas which may inactivate a penicillin by opening the b-lactam ring. However, some penicillins are considerably more resistant to this enzyme than others, and consequently may be extremely valuable in the treatment of infections

caused by b-lactamase producing bacteria. In general, the penicillins are active against Gram-positive bacteria;some members (e.g. amoxycillin) are also effective against Gram-negative bacteria, although not *Pseudomona aeruginosa*, whereas others (e.g. ticarcillin) are also active against this organism.

## PRODUCTION OF PENICILLIN BY FERMENTATION

Penicillin fermentation can be divided into **three phases**.
**First phase (trophophase)** - In this phase rapid growth occurs, lasts for about 30 hours during which mycelia are produced.
**Second phase (idiophase)** - It lasts for five to seven days; growth is reduced and penicillin is produced.
**Third phase** - carbon and nitrogen sources are depleted, antibiotic production ceases, the mycelia lyse releasing ammonia and the pH rises.

### Strain selection

In the early days of penicillin production, when the surface culture method was used, a variant of the original culture of *Penicillium notatum* discovered by Sir Alexander Fleming was employed. When however the production shifted to submerged cultivation, a strain of *Penicillium chrysogenum* designated NRRL 1951 (after Northern Regional Research Laboratory of the United States Department of Agriculture) discovered in 1943, was introduced. In submerged culture it gave a penicillin yield of up to 250 Oxford Units (1 Oxford Unit = 0.5988 of sodium benzyl penicillin) which was two to three times more than given by *Penicillium notatum.* A 'super strain' was produced from a variant of NRRL 1951 and designated X 1612. By ultraviolet irradiation of X-1612, a strain resulted and was named WISQ 176 after the University of Wisconsin where much of the stain development work was done. On further ultra violet irradiation of WISQ 176, BL3-D10 was produced, which produced only 75% as much penicillin as WISQ 176, but whose product lacked the yellow pigment the removal of which had been difficult. Present-day penicillin producing *P. chrysogenum* strains are far more highly productive than their parents. They were produced through natural selection, and mutation using ultra violet irradiation, x-irradiation or nitrogen mustard treatment. It was soon recognized that there were several naturally occurring penicillins, viz. Penicillins G, X, F, and K..
Penicillin G (benzyl penicillin) was selected because it was markedly more effective against pyogenic cocci. Furthermore, higher yields were achieved by supplementing the medium with phenylacetic acid, analogues (phenylalanine and phenethylaninie) of which are present in corn steep liquor used to grow penicillin in the United States. Present day penicillin-producing strains are highly unstable, as with most industrial
organisms, and tend to revert to low-yielding strains especially on repeated agar cultivation. They are therefore commonly stored in liquid nitrogen at – 196° or the spores may be lyophilized.
Penicillin has since been shown to be produced by a wide range of organisms including the fungi *Aspergillus, Malbranchea, Cephalosporium, Emericellopsis, Paecilomyces,*

*Trichophyton, Anixiopsis, Epidermophyton, Scopulariopsis, Spiroidium* and the actionomycete, *Streptomyces.*

**Medium and Inoculum Preparation**

The inoculum is usually built up from lyophilized spores or a frozen culture and developed through vessels of increasing size to a final 5-10% of the fermentation tank. As the antibiotic concentration in the fermentation beer is usually dilute the tanks are generally large for penicillin and most other antibiotic production. The fermentors vary from 38,000 to 380,000 liters in capacity and in modern establishments are worked by computerized automation, which monitor various parameters including oxygen content, Beta-lactam content, pH, etc. The medium for penicillin production now usually has as carbohydrate source glucose, beet molasses or lactose. The nitrogen is supplied by corn steep liquor. Cotton seed, peanut, linseed or soybean meals have been used as alternate nitrogen sources. The nitrogen source is sometimes exhausted towards the end of the fermentation and it must then therefore be replenished. Calcium carbonate or phosphates may be added as buffer. Sulfur compounds are sometimes added for additional yields since penicillin contains sulfur. The practice nowadays is to add the carbohydrate source intermittently, i.e. using fed-batch fermentation. Lactose is more slowly utilized and need not be added intermittently. Glucose suppresses secondary metabolism and excess of it therefore limits penicillin production. The pH is maintained at between 6.8 and 7.4 by the automatic addition of H2SO4 or NaOH as necessary.

Precursors of the appropriate side-chain are added to the fermentation. Thus if benzyl penicillin is desired, phenylacetic acid is added. Phenyl acetic acid is nowadays added continuously as too high an amount inhibits the development of the fungus. High yielding strains of *P. chrysogenum* resistant to the precursors have therefore been developed. Penicillin production is stimulated by the addition of surfactants in a yet unexplained mechanism. The temperature is maintained at about 25°C, but in recent times it has been found that yields were higher if adjusted according to the growth phase. Thus, 30-32°C was found suitable for the trophophase and 24°C for the idiophase. Aeration and agitation are vigorous in order to keep the components of the medium in suspension and to maintain yield in the highly aerobic fungus

**Extraction of penicillin after fermentation**

the separation of penicillin from them is based on the solubility, adsorption and ionic properties of penicillin. Since penicillins are monobasic carboxylic acids they are easily separated by solvent extraction as described below.

The fermentation beer or broth is filtered with a rotary vacuum filter to remove mycelia and other solids and the resulting broth is adjusted to about pH 2 using a mineral acid. It is then extracted with a smaller volume of an organic solvent such as amyl acetate or butyl acetate,

At the end of the fermentation the broth is transferred to a settling tank. Penicillin is highly reactive and is easily destroyed by alkali conditions (pH 7.5-8.0) or by enzymes. It is therefore cooled rapidly to 5-10°C. A reduction of the pH to 6 with mineral acids sometimes accompanied by cooling helps also to preserve the antibiotic. The fermentation broth contains a large number of other materials and the method used for keeping it at this very low pH for as short a time as possible. The aqueous phase is

separated from the organic solvent usually by centrifugation using Podbielniak centrifugal countercurrent separator. The organic solvent containing the penicillin is then typically passed through charcoal to remove impurities, after which it is back extracted with a 2% phosphate buffer at pH 7.5. The buffer solution containing the penicillin is then acidified once again with mineral acid (phosphoric acid) and the penicillin is again extracted into an organic solvent (e.g. amyl acetate). The product is transferred into smaller and smaller volumes of the organic solvent with each successive extraction process and in this way, penicillin becomes concentrated several times over, up to 80-100 times. When it is sufficiently concentrated the penicillin may be converted to a stable salt form in one of several ways which employ the fact that penicillin is an acid:
(a) it can be reacted with a calcium carbonate slurry to give the calcium salt which may be filtered, lyophilized or spray dried.
(b) it may be reacted with sodium or potassium buffers to give the salts of these metals which can also be freeze or spray dried;
(c) it may be precipitated with an organic base such as triethylamine.

**Administration;** When benzyl penicillin is administered intramuscularly it is given either as the sodium (or potassium) salt or as procaine penicillin. The former gives high blood levels but it quickly excreted. Procaine penicillin gives lower blood levels, but it lasts longer in the body because it is only slowly removed from the blood. It is produced by dissolving sodium or penicillin in procaine hydrochloride.

**Primary Modes of Action**

1. Cell wall synthesis inhibitors cell wall synthesis inhibitors generally inhibit some step in the synthesis of bacterial peptidoglycan. Generally they exert their selective toxicity against eubacteria because human cells lack cell walls

   Beta lactam antibiotics Chemically, these antibiotics contain a 4-membered beta lactam ring. They are the products of two groups of fungi, Penicillium and Cephalosporium molods, and are correspondingly represented by the penicillins and cephalosporins. The beta lactam antibiotics inhibit the last step in peptidoglycan synthesis, the final cross-linking between peptide side chains, mediated by bacterial carboxypeptidase and transpeptidase enzymes. Beta lactam antibiotics are normally bactericidal and require that cells be actively growing in

order to exert their toxicity. Examples of natural penicillins, such as Penicillin G or Penicillin V, are produced by fermentation of Penicillium chrysogenum. They are effective against Streptococcus, Gonococcus and Staphylococcus species, except where resistance has developed. They are considered narrow spectrum Semisynthetic penicillins first appeared in 1959. A mold produces the main part of the molecule (6-aminopenicillanic acid) which can be modified chemically by the addition of side chains. Many of these compounds have been developed to have distinct benefits or advantages over penicillin G, such as increased spectrum of activity (effectiveness against Gram-negative rods), resistance to penicillinase, effectiveness when administered orally, etc. Amoxycillin and Ampicillin have broadened spectra against Gram-negatives and are effective orally; Methicillin is penicillinase-resistant.

Cephalolsporins are beta lactam antibiotics with a similar mode of action to penicillins that are produced by species of Cephalosporium. They have a low toxicity and a somewhat broader spectrum than natural penicillins. They are often used as penicillin substitutes, against Gram-negative bacteria, and in surgical prophylaxis. They are subject to degradation by some bacterial beta-lactamases, but they tend to be resistant to beta-lactamases from Staphylococcus aureus.

2. Cell membrane inhibitors disorganize the structure or inhibit the function of bacterial membranes. The integrity of the cytoplasmic and outer membranes is vital to bacteria, and compounds that disorganize the membranes rapidly kill the cells. However, due to the similarities in phospholipids in eubacterial and eukaryotic membranes, this action is rarely specific enough to permit these

compounds to be used systemically. The only antibacterial antibiotics of clinical importance that acts by this mechanism is Polymyxin, produced by Bacillus polymyxas. Polymyxin is effective mainly against Gram-negative bacteria and is usually limited to topical usage. Polymyxins bind to membrane phospholipida and thereby interfere with membrane function. Polymyxin is occasionally given for urinary tract infections caused by Pseudomonas species that are gentamicin, carbenicillin and tobramycin resistant.

3. Protein synthesis inhibitors Many therapeutically useful antibiotics owe their action to inhibition of some step in the complex process of translation. Their attack is always at one of the events occurring on the ribosome and rather than the stage of amino acid activation or attachment to a particular tRNA. Most have an affinity or specificity for 70S (as opposed to 80S) ribosomes, and they achieve their selective toxicity in this manner. The most important antibiotics with this mode of action are the tetracyclines, chloramphenicol, the macrolides (e.g. erythromycin) and the aminoglycosides (e.g. streptomycin).

` The aminoglycosides are products of Streptomyces species and are represented by streptomycin, kanamycin, tobramycin and gentamicin. These antibiotics exert their activity by binding to bacterial ribosomes and preventing the initiation of protein synthesis. Aminoglycosides have been used against a wide variety of bacterial infections caused by Gram-positive and Gram-negative bacteria. Streptomycin has been used extensively as a primary drug in the treatment of tuberculosis. Gentamicin is active against many strains of Gram-positives and Gram negative bacteria, including some strains of Pseudomonas aeruginosa.

Kanamycin (a complex of three antibiotics, A, B and C) is active at low concentrations against many Gram-positive bacteria, including penicillin-resistant staphylococci. Gentamicin and Tobramycin are mainstays for treatment of Pseudomonas infections.

The tetracyclines consist of eight related antibiotics which are all natural products of Streptomyces, although some can now be produced semisynthetically. Tetracycline, chlortetracycline and doxycycline are the best known. The tetracyclines are broad-spectrum antibiotics with a wide range of activity against both gram-positive and Gram-negative bacteria. The tetracyclines act by blocking the binding of aminoacyl tRNA to the A site on the ribosome. Tetracyclines inhibit protein synthesis on isolated 70S or 80S (eukaryotic) ribosomes, and in both cases, their effect is on the small ribosomal subunit. However, most bacteria possess an active transport system for tetracycline that will allow intracelluylar accumulation of the antibiotic at concentrations 50 times as great as that in the medium. This greatly enhances its antibacterial effectiveness and accounts for its specificity of action, since an effective concentration cannot be accumulated in animal cells. Thus a blood level of tetracycline which is harmless to animal tissues can halt protein synthesis in invading bacteria.

The Macrolides are a family of antibiotics whose structures contain large lactone rings linked through glycoside bonds with amino sugars. The most important members of the group are erythromy6cin and oleandomycin. Erythromycin is active against most Gram-positive bacteria, Neisseria, legionella and Haemophilus species, but not against the Enterobacteriaceae. Macrolides inhibit

bacterial protein synthesis by binding to the 50S ribosomal subunit. Binding inhibits elongation of the protaein by peptidyl transferase or prevents traslocation of the ribosome or both. Macrolides are bacteriostatic for most bacteria but are bacteriocidal for a few Gram-positive bacteria.

4. Effects on Nucleic Acids Some chemotherapeutic agents affect the synthesis of DNA or RNA, or can bind to DNA or RNA so that their messages cannot be read. Either case, of course, can block the growth of cells. The majority of these drugs is unselective, however, and affects animal cells and bacterial cells alike and therefore has no therapeutic application. Two nucleic acid sysnthesis inhibitors which have selective activity against prokaryotes and some medical utility are nalidixic acid and rifamyceins.

5. Competitive Inhibitors The competitive inhibitors are mostly all synthetic chemotherapeutic agents. Most are “growth factor analogs” which are structurally similar to a bacterial growth factor but which do not fulfill its metabolic function in the cell. Some Sulfonamides were introduced as chemotherapeutic agents by Domagk in 1935, which showed that are bacteriostatic and some are bactericidal. One of these compounds (prontosil) had the effect of curing mice with infections caused by beta-hemolytic streptococci. Chemical modifications of the compound sulfanilamide gave compounds with even higher and broader antibacterial activity. The resulting sulfonamides have broadly similar antibacterial activity, but differ widely in their pharmacological actions. Bacteria which are almost always sensitive to the sulfonamides include Streptococcus pneumonia, beta-hemolytic streptococci and Escherichia. coli. The sulfonamides have been

extremely useful in the treatment of uncomplicated UTI caused by E. coli, and in the treatment of meningococcal meningitis (because they cross the blood-brain barrier).

| Chemical class | Examples | Biological source | Spectrum (effective against) | Mode of action |
|---|---|---|---|---|
| Beta-lactams (penicillins and cephalosporins) | Penicillin G, Cephalothin | Penicillium notatum and Cephalosporium species | Gram-positive bacteria Staphylococcus and Streptococcus species | Inhibits steps in cell wall (peptidoglycan) synthesis and murein assembly |
| Semi synthetic penicillin | Ampicillin, Amoxycillin | | Gram-positive and Gram-negative bacteria | Inhibits steps in cell wall (peptidoglycan) synthesis and murein assembly |
| Aminoglycosides | Streptomycin | Streptomyces griseus | Gram-positive and Gram-negative bacteria | Inhibit translation (protein synthesis) |
| Glycopeptides | Vancomycin | Streptomyces orientales | Gram-positive bacteria, esp. Staphylococcus aureus | Inhibits steps in murein (peptidoglycan biosynthesis and assembly |
| Macrolides | Erythromycin | Streptomyces erythreus | Gram-positive bacteria, Gram-negative bacteria not enteric, Neisseria, legionella, Mycoplasma species | Inhibits translation (protein synthesis) |
| Polypeptides | Polymyxin | Bacillus polymyxas | Gram-negative | Damages cytoplasmic |

| | | | | |
|---|---|---|---|---|
| | | | bacteria e.g. Escherichia coli | membranes |
| Tetracyclines | Tetracycline | Streptomyces species | Gram-positive and Gram-negative bacteria, Rickettsias | Inhibit translation (protein synthesis) |

## 2.0 The Challenge of Antibiotic Resistances

Bacteria inhibit a global environmental pool in which resistant bacteria, and genes transferring antibiotic resistance between bacteria, can and do spread easily between people and animals. A continuous process of exchange of genes takes place within the microbial world. The two variable factors affecting the spread of antibiotic resistance are the selection pressure exerted by antibiotic use, and the ease with which resistant organisms are able to spread between people by "cross-infection".

Challenges of antibiotic resistance include the following;

- Enormous growth of global trade and travel.
- Evolution of microorganism.
- Aids epidemic, has increased the population of immunocompromised patient
- Increase in cost in health care in developing countries.
- Containment of antibiotic resistant strains in food-producing animals.
- Illegal licensing, distribution and sales of Antimicrobial agents.
- Poor monitoring of global emergences of resistance pathogen in hospital and communities.

- Low quality assurance of laboratory test of antibiotics.
- Urbanization with its associated overcrowding and poor sanitation.
- Environmental pollution
- Societal Factors.

## 2.1 Factors Causing Acquired Bacteria Resistances.

1. Misuse of antibiotics by physicians in clinical practice
2. Misuse of antibiotics by unskilled practitioners
3. Misuse of antibiotics by the public
4. Lack of Quality Compliance and Monitoring
5. Degraded Antibiotics
6. Expired Antibiotics
7. Counterfeit Drugs
8. Adulterated Drugs
9. Crowding and Unhygienic Conditions
10. Inadequate Hospital Infection Control Practices
11. Susceptibility Testing and Surveillance
12. Defective Antibiotic Susceptibility Assays
13. Economic and Political Factors

**Types of Bacterial Resistance to Antibiotics:**

The basis of bacterial resistance to antibiotics could be

- Inherent (Natural) Resistance : Bacteria may be inherently resistant to an antibiotic. For example, a streptomycete has some gene that is responsible for resistance to its own antibiotic; or a Gram-negative bacterium has an outer membrane that establishes a permeability barrier against the antibiotic; or an organism lacks a transport system for the antibiotic; or it lacks the target or reaction that is hit by the antibiotic.

- Acquired Resistance: Bacteria can develop resistance to antibiotics, e.g. bacterial population's previously-sensitive to antibiotics become resistant. This type of resistance results from changes in the bacterial genome. Acquired resistance is driven by two genetic processes in bacteria;

(1) Mutation and selection (sometimes referred to as vertical evolution)

(2) Exchange of genes between strains and species (sometimes called horizontal evolution).

1. Vertical evolution is strictly a matter of Darwinian evolution driven by principles of natural selection; a spontaneous mutation in the bacterial chromosome imparts resistance to a member of the bacterial population. In the selective environment of the antibiotic, the wild type (non mutants) is killed and the resistant mutant is allowed to grow and flourish. The mutation rate for most bacterial genes is approximately $10^{-8}$. This means that if a bacterial population doubles from $10^8$ to $2x10^8$ cells, there is likely to be a mutant present for any given gene. Since bacteria grow to reach population densities far in excess of 109 cells; such a mutant could develop from a single generation during 15 minutes of growth.
2. Horizontal evolution is the acquisition of genes for resistance from another organism. For example, a streptomycete has a gene for resistance to streptomycin (its own antibiotic), but somehow that gene escapes and gets into E. coli or Shigella. Or, more likely, some bacterium develops genetic resistance through the process of mutation and selection and then donates these genes to some other bacterium through one of several processes for genetic exchange that exist in bacteria.

Bacteria are able to exchange genes in nature by three processes; conjugation, transduction and transformation. Conjugation involves cell-to-cell contact as DNA crosses a sex pilus from donor to recipient. During transduction, a virus transfers the genes between mating bacteria. In transformation, DNA is acquired directly from the environment, having been released from another cell. Genetic recombination can follow the transfer of DNA from one cell to another leading to the emergence of a new genotype (recombinant). It is common for DNA to be transferred as plasmids between mating bacteria. Since bacteria usually develop their genes for drug resistance to other strains and species during genetic exchange processes.

The combined effects of fast growth rates, high concentrations of cells, genetic processes of mutation and selection, and the ability to exchange genes, account for the extraordinary rates of adaptation and evolution that can be observed in the bacteria. For these reasons bacterial adaptation (resistance) to the antibiotic environment seems to take place very rapidly in evolutionary time: bacteria evolve fast!

Conditions for acquisition of resistance by microorganisms

(i) If the bacteria can inactivate the drug before it reaches its target within the bacterial cell.

(ii) If the outer layers of the cell are impermeable, and prevent the drug from entering.

(iii) If the drug enters but is then pumped back out again (“efflux”).

(iv) If the target is altered so that it is no longer recognized by the antibiotic, or

(v) If the bacteria acquire an alternative metabolic pathway that renders the antibiotics target redundant (“by-pass”).

`3.2 Mechanisms of antibiotics resistance

1. Pumping Out the Antibiotic

Efflux pumps are transport proteins that can provide innate resistance to antibiotics by expelling toxic substances into the extracellular environment. These proteins can be associated with multiple drug resistance as they often transport antibiotics of distinct structural classes.

A large proportion of transport genes found in bacteria encode for efflux pumps. Their expression can be part of an operon system, a genetic for multiple drug resistance. Regardless, their elevated level of expression in highly resistant clinical strains should not be ignored. For example, resistance to bile salts and some antibiotics in Escherichia coli has been associated with over expression of acrAB, an energy dependent efflux mechanism found in the bacteria.

2. Enzymatic Modifications

Antibiotic-modifying enzymes inactivate antibiotics by chemically altering their structure. This mode of resistance is very specific in comparison to other mechanisms. A classic example is the hydrolysis of the beta-lactam ring by beta-lactamases in penicillin and cephalosporin. Anther predominant example is the resistance to aminoglycoside drugs brought about by enzymatic inactivation of drug activity. Such enzymes can acetylate the amino group of an antibiotic, preventing the addition of other chemical ligands.

3. Modification of the Antibiotic Target

This mode of antibiotic resistance can occur through mutations, altering key binding elements, such as ribosomal RNA, or by reprogramming the target. For example, a mutatikon in the penicillin binding protein (PBP) can lower the affinity of penicillin towards PBPs, conferring bacterial resistance to beta-lactam antibiotics. Similar mechanisms have been observed in erythromycin resistance pathways.

| Antibiotic | Mechanism | Organisms |
|---|---|---|
| Penicillin, ampicillin, Carbenicillin, Oxacillin, methicillin, cephalosporin | Beta-lactamase hydrolysis | Staphylococci, Enterococci, Enterobacteriaceae, Pseudomonas, Bacteroides species |
| Chloramphenicol | Acetylation | Staphylococci, Enterococci, Streptococci, Enterobacterianceae, Pseudomonas species |
| Tetracyclines | Permeability block | Staphylococci, Enterococci, Streptococci, Enterobacteriaceae, Pseudomonas, Bacteroides species |
| Aminoglycosides: Streptocycin Neomycin, kanamycin Tobromycin, Amikacin<br><br>Macrolides-linconoids Erythromycin, Clindermycin<br>Trimethoprin<br>Sulfonamides<br>Fostomycycin<br>Vancomycin | Acetylation, phosphorylation Adenylation<br><br>Altered 23S RNA<br><br>Altered dihydrofolate reductase<br><br>Altered tetraphydropteroic synthetase | Staphylococci, Enterococci, Enterobacteriaceae, Pseudomonas. Species<br>Staphylococci, Enterococci, Streptococci, Bacteroides species<br>Staphylococci, Enterobacteriaceae,<br>Staphylococci, Enterococci, Streptococci, Enterobacteriaceae, |

| | | |
|---|---|---|
| | Altered glucose<br>New protein | Pseudomonas. Species<br>Staphylococci species<br>Enterobacteriaceae,<br>Enterococci |

4.0 Combating the Problem of Antibiotic Resistance

The recommendations of World Health Organization for ensuring proper drug use can be adapted to combat the escalation of community-acquired antibiotic resistance in developing countries. The misuse of antibiotics by health-care professionals, unskilled practitioners, and patients can be alleviated by auditing antibiotics, limiting antibiotic choice, developing prescription guidelines, and emphasizing continuing medical and public education. The quality of antibiotics can be improved by emphasizing quality compliance and monitoring antimicrobial drugs manufactured or dispensed. Such reforms will help control substandard drugs that are degraded, counterfeit, or bioinequilvalent. Dissemination of resistant organisms in the community can be impeded by improved public sanitation and hygienic practices and upgraded hospital infection control. Finally, strategies to ensure that these recommendations are adopted and implemented under difficult economic and political conditions can be formulated. Antimicrobial resistance will continue to escalate in developing countries unless corrective measures are instituted.

1. Prudent use in human medicine
2. Prudent use in animals
3. Infection control

4. Surveillance
5. New drug development
6. International
7. Resources for research and data-collection

### 4.1 Alternatives to Antibiotics

1. Bacterial interference, also known as bacteriotherapy, is the practice of deliberately inoculating hosts with nonpathogethic (commensal) bacterial to prevent infection by pathogenic strains. To establish an infection and propagate disease, pathogenic bacteria must find nutrients and attachment sites (adhesion receptors). Infection by pathogenic bacteria is prevented by commensal bacteria, which compete with pathogenic bacteria for nutrients and adhesion receptors or spur attack through secretion of antimicrobial compounds.

This treatment has had promising results in infections of the gut, urogential tract, and wound sites. The major advantage of using bacteria in a positive way to benefit health, known as “probiotic” usage, is avoided without stimulating the host’s immune sytem and decreases selection for antiobiotic resistance (Sean, 2004).

**2. Bateriophage Therapy**

Bacteriophages (commonly called “phages”) are viruses that infect bacteria and were recognized as early as 1896 as natural killers of bacteria (Summer 2001). Bacteriophages take over the host’s protein-making machinery, directing the host bacteria to make viral proteins of their own. Therapeutically, Bacteriophages were used as a prophylaxis against cholera, typhoid fever, and dysentery.

Bacteriophage therapy is quite attractive for the following reasons:

- Phage particles are narrow spectrum agents, which mean they possess an inherent mechanism to not only infect bacteria but specific strains.
- Other pathogens may be targeted through manipulation of phage DNA.
- Exponential growth and natural mutational ability make bacteriophage great candidates for thwarting bacterial resistance.

### 3. Bacterial vaccines

Development of bacterial vaccines has become an increasingly popular idea with the advent of complete genomic sequencing and the understanding of virulence regulatory mechanisms. Vaccine do not suffer the problem of resistance because a vaccine enhances the body's natural defenses, while an antibiotic operates separately from the body's normal defenses.

Anti-staphylococcal vaccines have shown limited efficacy, because of immunological variation between ***Staphylococcus*** species, and limited duration of effectiveness of the antibodies produced.

Bacterial genomics allows scientists to scan an entire bacterial genome for specific sequences that may be used to stimulate a protective immune response against specific bacterial strains. This approach expedites the drug discovery process and, more importantly, provides a more rational, target-based approach.

### 4. Cationic peptides

These diverse peptides are natural compounds that poses both hydrophobic and hydrophilic characteristics, which means portions of the molecule are water avoiding or water loving. Cationic peptides are found throughout nature in the immune systems of bacteria, plants, invertebrates, and vertebrates.

Cationic peptides have several mechanisms of actions, all of which involve interaction with the bacterial cell membrane leading to cell death. From a therapeutic standpoint, these proteins have great promise, as they have coevolved with commensal bacteria yet have maintained the ability to target pathogenic bacteria.

**5. Cyclic D, L-a-peptides**

Unlike cationic peptides, cyclic D, L-a-peptides are synthetic and amphipathic (molecules having both water loving and water hating characteristics) cell membrane disruptors. As the name implies these peptides are cyclic in nature and are composed of alternating D and L amino acids, Cyclic D, L-a-peptides are engineered to target gram-positive and negative membranes (not mammalian cell membranes). In contrast to any other known class of peptides, these peptides can self-assemble in flat ring shaped conformations forming structures known as nanotubes, which specifically target and puncture bacterial cell membranes resulting in rapid cell death.