

IMMUNOLOGY AND MEDICAL MICROBIOLOGY

Antimicrobial Chemotherapy

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17-May-2006 (Revised 30-Jan-2007)

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Keywords

Antibiotics, Bactericidal, Bacteriostatic, Effects of antimicrobial agents, Bacterial cell wall synthesis, Cell membranes, DNA synthesis, Protein synthesis, Antibiotic resistance.

Introduction

Pathogenic microbes have a history of co-existence with animals, as well human beings. As early as hundred years back humans, did not have any effective measures and means to combat pathogenic microorganisms. The medical practices were primarily aimed at palliative measures to alleviate the symptoms of the disease than attacking the source of infectious diseases. Robert Koch and Henle proposed and tested a set of postulates known as germ theory of diseases that implied that there is a specific causative agent of each disease. Presently we are aware that the list of such causative pathogenic entities is large and covers bacteria, viruses, fungi, protozoa, viroids, virusoids and prions. Joseph Lister noted that if moulds were present in the cultures, the bacteria in these cultures appeared non-motile and degenerate; whereas if bacteria grew without moulds, they were highly motile. Lister concluded that moulds produced a substance or substances that adversely affected the viability of bacteria. He then reasoned that culture filtrates obtained from moulds should prevent infection if used to wash surgical wounds. This practice started sixty years before Alexander Fleming described the antibacterial properties of penicillin, produced from a mould that he had originally misidentified.

The problem of producing sufficient amount of antibiotic from mould cultures however, defeated both Lister and Fleming. Indeed, Fleming was slow to appreciate the clinical applications of his observations. He thought that penicillin may be used as a selective agent in laboratory media rather than to be administered to patients directly. It was not until the early days of the Second World War that an allied Anglo-American effort overcame the problem of large-scale production and penicillin therapy for human infection was introduced. By the end of the war, penicillin was so plentiful that it was being used to cure cases of gonorrhoea in the allied troops. After the war, penicillin became generally available. This was however not so for the first person who was treated with penicillin - a policeman with overwhelming staphylococcal sepsis. The antibiotic was in such short supply that it had to be re-purified from the patient's urine. In this instance, although the antibiotic caused relief from clinical symptoms, once the supply of penicillin ran out, although the staphylococcal infection regained its hold and the patient died. Penicillin represented the first true antibiotic: a substance produced by one microorganism that, in very small amounts, inhibits or kills other microorganisms.

Antibiotics are the products of microbes which in dilute solution, inhibit or kill other organisms. Antimicrobial agents include antibiotics and synthetic compounds that have the same effect. Naturally occurring antibiotics may be modified to give semisynthetic derivatives. These often differ from the parent compound in antimicrobial activity or pharmacological properties. The term 'antibiotic' is often applied very loosely and includes synthetic antibacterial agents as well, although this is strictly incorrect. The agents that kill bacteria are called bactericidal and those whose effects are reversible upon removal of the drug are bacteriostatic. The graphs below show the growth curves of a bacterium treated with two drugs (Fig. 1). The left profile shows the activity of a bacteriostatic drug. The bacterial growth resumes when the drug is withdrawn. The cidal drug kills bacteria as depicted in the graph. These terms, -cidal and -static, are used to describe the action of disinfectants as well as antibiotics. For example, chloramphenicol is bacteriostatic and gentamicin is bactericidal; phenol is germicidal whereas mercury ions are bacteriostatic. By definition, disinfectants are the compounds that are applied to the non-animated surfaces, and antiseptics are the compounds that are applied to the animated surfaces to kill/ or inactivate the pathogenic microorganisms. Joseph Lister was the first to use carbolic acid

(commonly known as phenol) as an antiseptic to swab the surgical wounds. This reduced the human fatalities drastically because of the control of infections which used to follow most surgical operations.

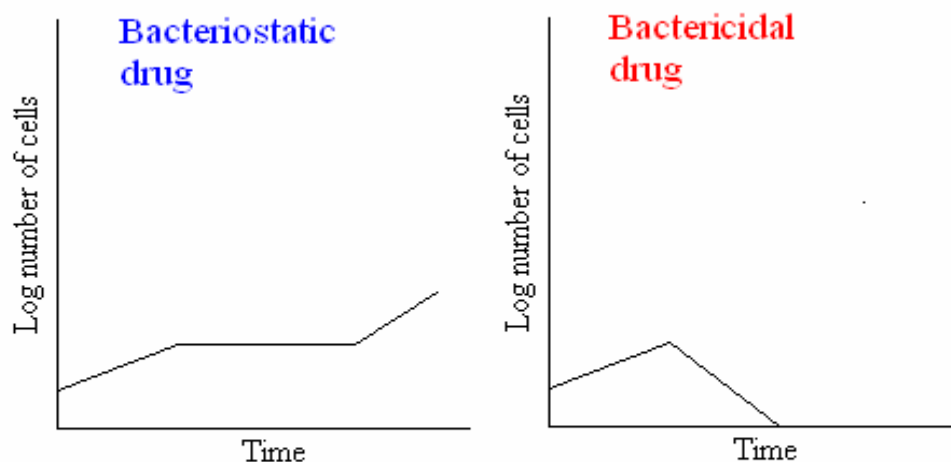


Fig. 1: Effect of a bacteriostatic and a bactericidal drug on the microbial population

The invention of chemotherapy by Paul Ehrlich, a German physician provided a tool to combat the microbial diseases. He was astonished to see that certain acidic or basic dyes were able to stain specific parts of the cells in histological specimens. He thought that there might be certain compounds/ dyes that might specifically bind pathogenic microorganisms but not to human or animal cells. His efforts to find such a 'magic bullet' was finally rewarded when his assistant, Sahachiro Hata in 1890 found that 606th compound they tested was active against syphilis, a sexually transmitted diseases. The disease is transmitted through sexual contact and may prove fatal in its later stages. This compound was referred as Salvarsan, which however possessed significant toxicity for humans. This discovery laid the foundations of chemotherapy and impetus to search for newer compounds - naturally occurring or synthetically made with specificity for pathogenic microorganisms. One of the greatest triumphs of modern medicine has been the introduction of a rational system of antimicrobial chemotherapy to combat infectious diseases. Since time immemorial, folk remedies have exploited moulds or mould extracts to treat infections. In the early days of microbiology, attempts were made to use extracts derived from fungal cultures to prevent surgical wound infection. Subsequent advancement in chemotherapy did not occur until 1930s when Gerhardt Domagk, working in a German company involved in the manufacture of dyes and greatly influenced by Ehrlich's earlier ideas, tested various synthetic dyes as antimicrobial agents. He found that Prontosil was effective in the treatment of experimental streptococcal infections in mice. In the coming years, French scientists discovered

that the active moiety in Prontosil was the colorless compound sulfanilamide (Fig. 2). Many sulfanilamide derivatives called as sulfa drugs were developed later and some are even used today to treat microbial infections along with other newer antimicrobial compounds. The development of antibiotics occurred in parallel with the search for chemical antibacterial agents: artificial compounds that inhibited or killed microbes. The most successful of the early antimicrobial compounds, the sulphonamides, are still in use today.

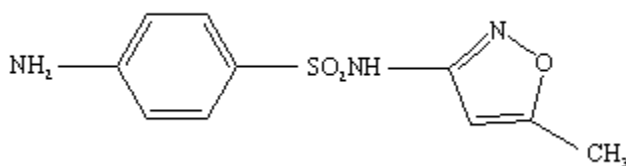


Fig. 2: Chemical structure of sulphamethoxazole, a sulfanilamide

Bacteria are good targets for the activity of antimicrobial substances. Being prokaryotes, their metabolic pathways are significantly different from that of humans. Antibiotics may act upon bacterial reactions that are not found in human cells. This provides the basis for the **selective toxicity** of antibiotics, affecting the bacteria but not the human host. Not all antibiotics are without their side effects. The adverse effects of antibiotics are not necessarily associated with their antimicrobial properties. For example, penicillin allergy is very common in human. Penicillin allergy is due to the presence of the thiazolidine ring of penicillin (Fig. 3). β -lactam ring rather than the thiazolidine ring is however responsible for the antibiotic activity. Fungi and protozoa have a metabolism that is much closer to that of humans than do bacteria. Moreover, viruses are obligate intracellular parasites that depend almost exclusively upon human metabolism for their replication. Consequently, anti-virus, antifungal and anti-protozoan drugs are more limited in their scope and are generally more toxic to humans than are antibacterial drugs.

Antibiotics

In the laboratory, susceptibility is often measured using a disk diffusion test. Antibiotic solutions of particular concentrations are dried onto filter paper disks. These are then applied to a mat of the microbes under test that has been inoculated previously on an appropriate solid medium (Mueller-Hinton Agar). In the Stokes controlled sensitivity test, a control organism is inoculated on part of the plate and the test organism is plated on the remainder (Fig. 4). Disks are placed at the interface and the zones of inhibition are compared. The use of a sensitive control shows that the antibiotic is active. So if the test organism grows up to the disk it may safely be assumed that the test organism is resistant to that drug.

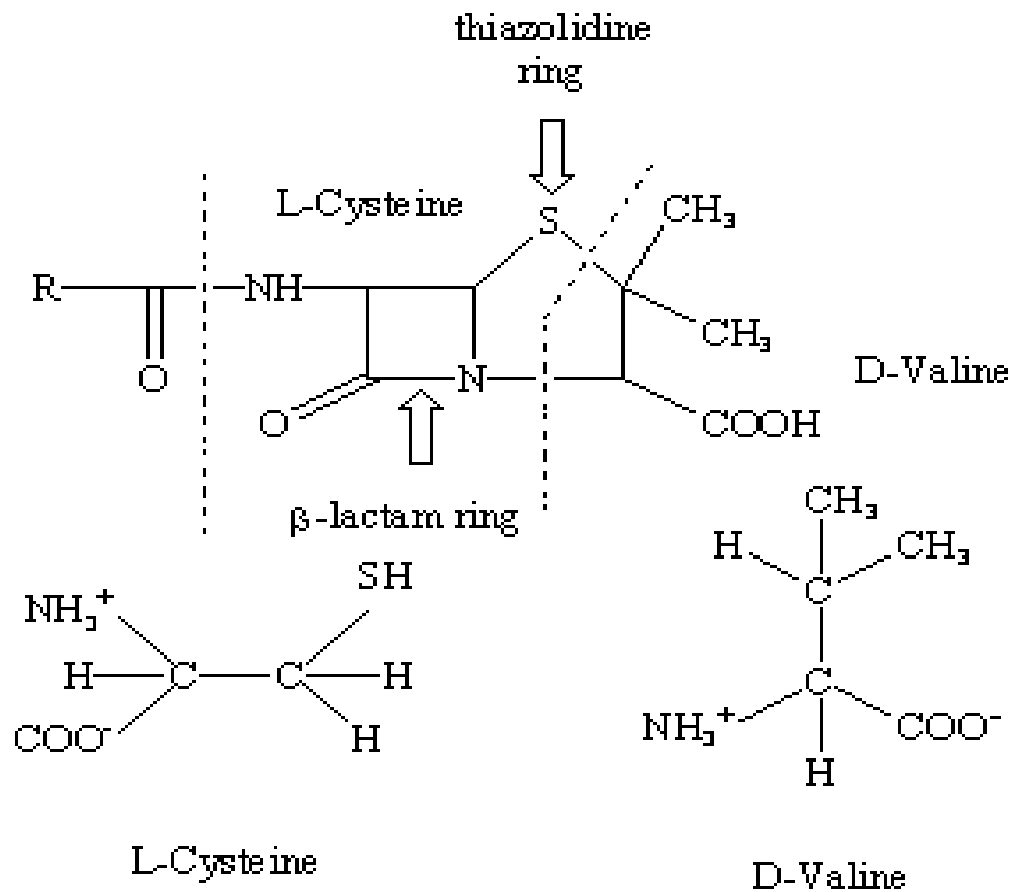


Fig. 3: The penicillin nucleus showing its amino acid structure and indicating β -lactam and thiazolidine rings

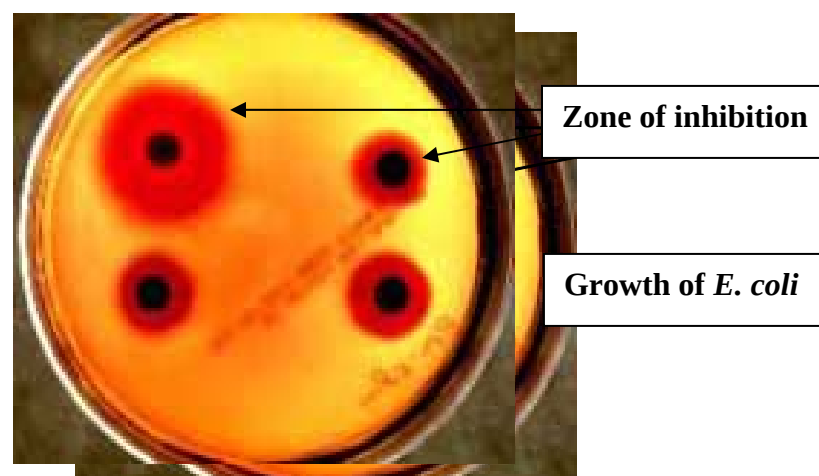


Fig. 4: Stokes' sensitivity test

An alternative measure of susceptibility is to determine the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of a drug or antibiotic. A series of broth are mixed with serially diluted antibiotic solutions and a standard inoculum is applied (Fig. 5). After incubation, the MIC is the lowest concentration at which growth of the organism has been inhibited. The more resistant an organism is, the higher will be the MIC. The MBC is measured by inoculating the broth used for MIC determinations onto drug-free medium. The MBC is the lowest concentration at which no visible growth is observed. Cidal drugs have MBC values that are close to the MIC value for particular organisms. With static agents, the MIC is much lower than the MBC. Once the bacteria are removed from the drug, they can grow on drug-free medium at most concentrations.

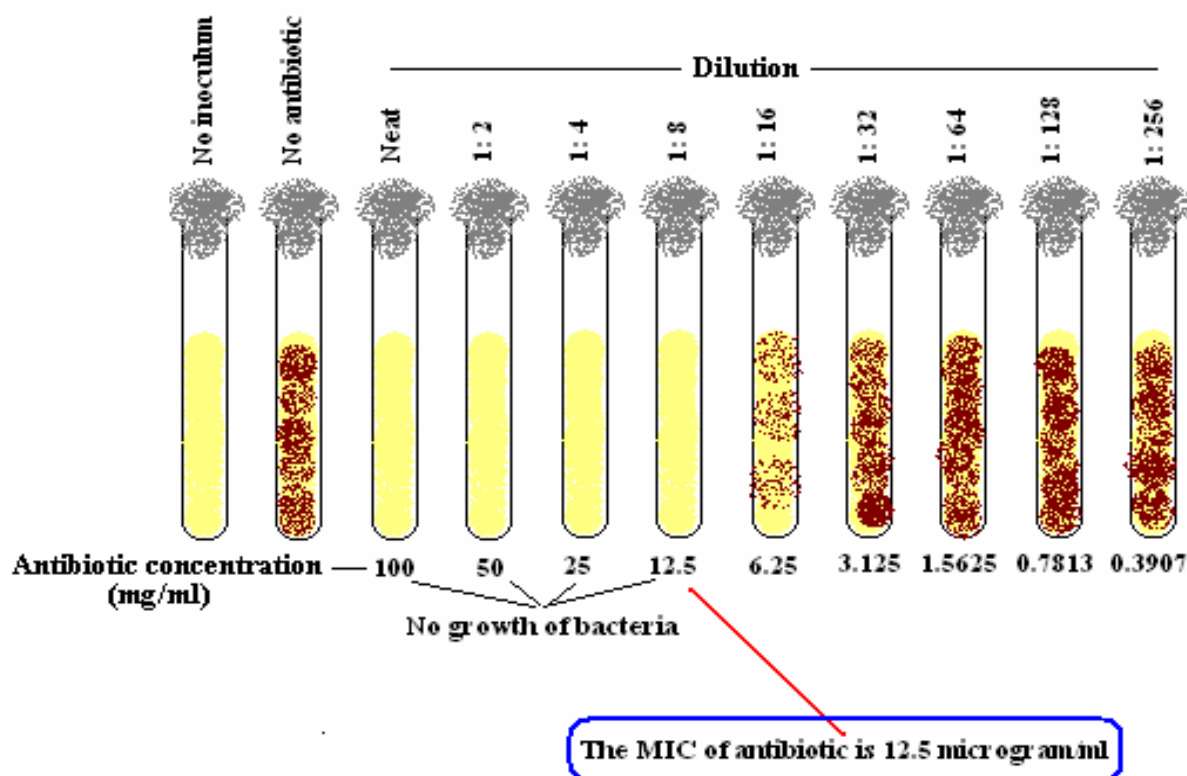


Fig. 5: The MIC/MBC test of a bactericidal drug. The test tubes containing sterile broth without antibiotic, and with two-fold serially diluted antibiotic are inoculated uniformly with a bacterial culture. The growth of the organism is observed after 24-48 h of incubation at optimal temperature (usually 35°C)

Mostly antibiotics are administered as single agents. There are, however, occasions when two or more drugs are used in combination. Antimicrobial agents may affect each other when used in combination. The effect may simply be additive. In some cases the activity of one drug enhances that of a second drug. This is referred to as synergy. Alternatively, drugs may interfere with each other as in antagonism. Penicillins and bacteriostatic drugs such as tetracyclines are antagonistic; since penicillins require actively growing cells and static drugs prevent cell growth. In contrast,

aminoglycosides are synergistic when used in combination with penicillins. This is important when considering antimicrobial therapy for conditions such as endocarditis. In this condition, it is essential that the antimicrobial regimen be bactericidal as the bacteria become walled off inside vegetations. Synergistic combinations are typically used to treat this condition (Fig. 6).

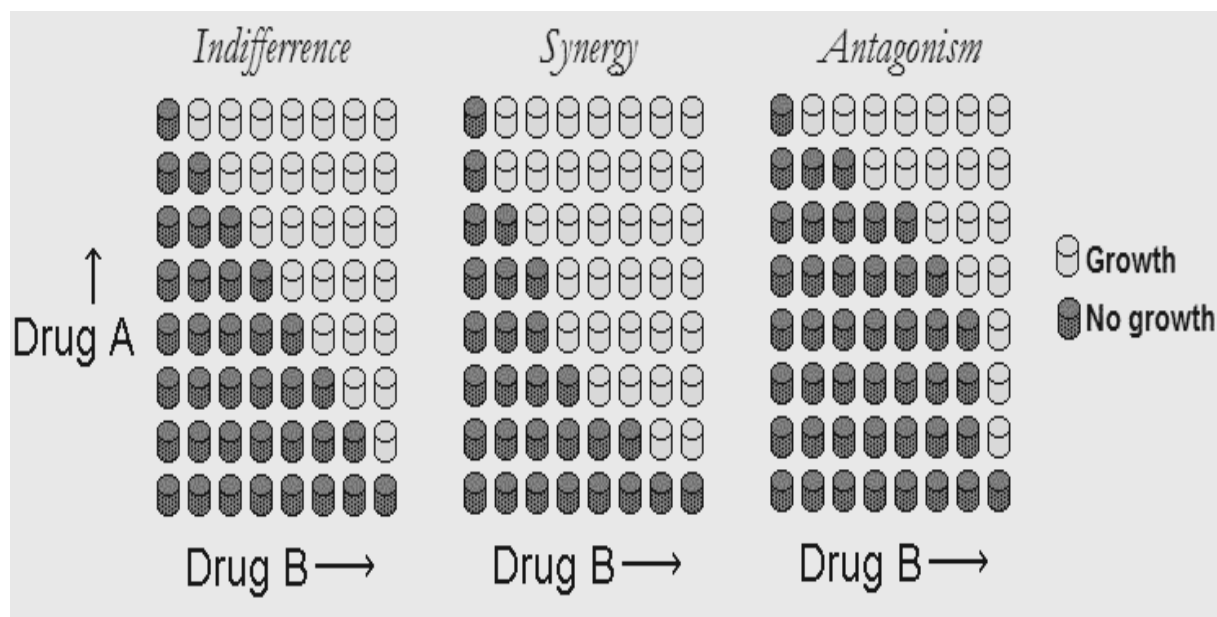


Fig. 6: The synergistic and antagonistic action of two drugs A and B. Activity of drug A enhances that of a second drug B, which is referred to as synergy. Alternatively, drug A and B may interfere with each and this effect is referred as antagonism

Effects of combining antimicrobial agents

Although, as illustrated above, laboratory tests *in vitro* can test for synergy or antagonism this effect is not necessarily apparent when combinations are used *in vivo*. Sulphonamides and trimethoprim both act on folic acid metabolism and show synergistic activity against bacteria *in vitro* but it is difficult to achieve a synergistic ratio of these drugs in humans. The antimicrobial agents exert bactericidal or bacteriostatic effects by inhibiting certain metabolic pathways that are vital for the growth of the microorganisms. The antibiotics exert their bactericidal or bacteriostatic effect(s) on the target organisms by the following methods:

- Inhibition of bacterial cell wall synthesis
- Inhibition of bacterial cell membrane functions
- Inhibition of DNA synthesis
- Inhibition of RNA synthesis
- Inhibition of protein synthesis

There are a few other antibiotics that are capable of inhibiting the growth or cause the death of fungi, protozoa and inactivation of viral synthesis. Nucleic acid analogues (purines or pyrimidines) are choicest compounds used to treat viral infection in human and animals. However, many of these compounds possess marked toxicity and certain serious side effects.

i) Inhibition of bacterial cell wall synthesis

Murien or peptidoglycan is an exclusive bacterial polymer and so potentially should provide an excellent target for selective chemotherapy (Fig. 7). Unfortunately, not all the intermediate steps in peptidoglycan biosynthesis are confined to bacteria and some antimicrobials that inhibit such reactions may be very toxic to humans as well as to bacteria. Peptidoglycan is unique among biological polymers because it contains both L- and D-isomers of its constituent amino acids. Antibiotics may act at several stages during peptidoglycan synthesis. Some are valuable chemotherapeutic agents; others are too toxic for human use.

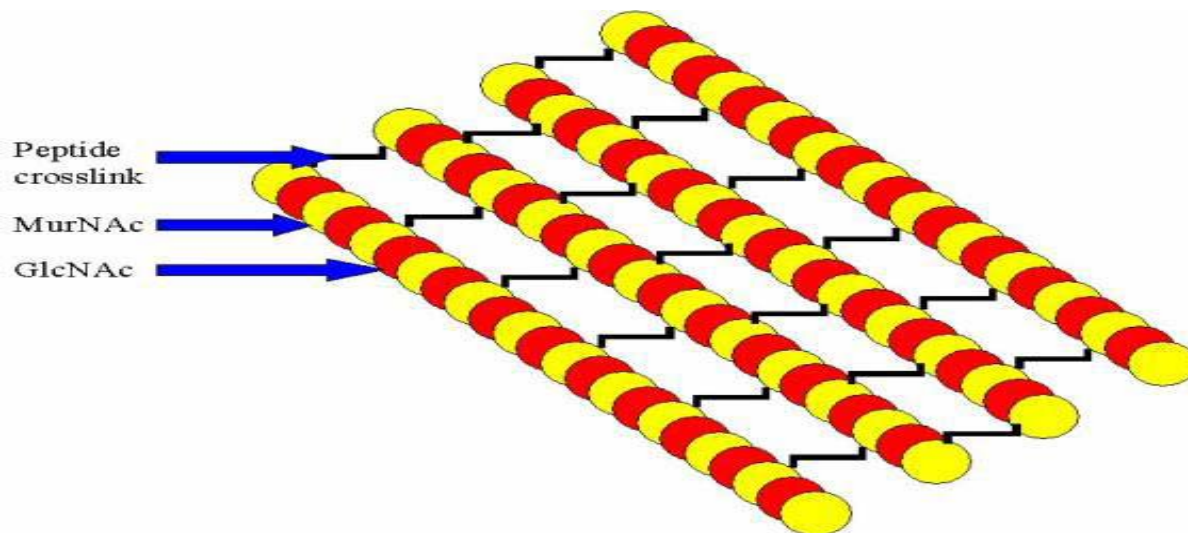


Fig. 7: Structure of bacterial peptidoglycan comprising a backbone of alternate units of N-acetyl muramic acid (MurNAc) and N-acetyl glucosamine (GlcNAc) cross

β -lactams

The β -lactam group of antibiotics includes an enormous diversity of natural and semi-synthetic compounds that inhibit several enzymes associated with the final step of peptidoglycan synthesis. All the members of this enormous family are derived from a β -lactam structure: a four-member ring in which the β -lactam bond resembles a peptide bond (Fig. 8a-d). The multitude of chemical modifications based on this four-member ring permits the astonishing array of antibacterial and pharmacological properties within this valuable family of antibiotics. Clinically useful β -lactam compounds include the penicillins, cephalosporins, monobactams and carbapenems. Many new variants of the β -lactam ring are currently being explored. Certain β -lactams have limited use directly as therapeutic agents, but may be used in combination with other β -lactams to act as β -lactamase inhibitors. Co-amoxyclav, for example is a combination of amoxycillin and the β -lactamase inhibitor clavulanic acid. During cross-linking of the peptidoglycan polymer, one (terminal) D-alanine residue is cleaved from the peptidoglycan precursor and β -lactam drugs prevent this reaction. More recent studies have shown that the activity of this class of drugs is more complicated and involves other processes as well as preventing cross-linking of peptidoglycan.

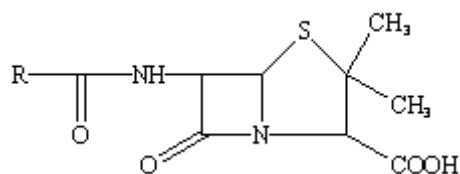


Fig. 8a: Chemical structure of a penicillin nucleus

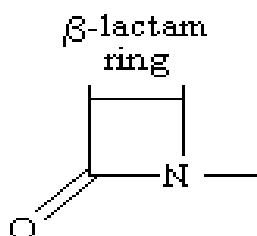


Fig. 8b: Chemical structure of a monobactam nucleus

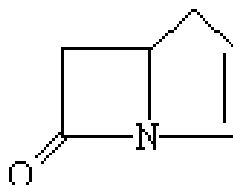


Fig. 8c: Chemical structure of a carbapenem nucleus

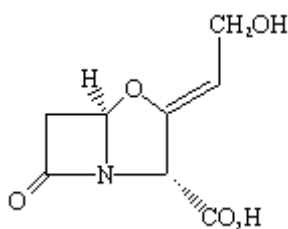
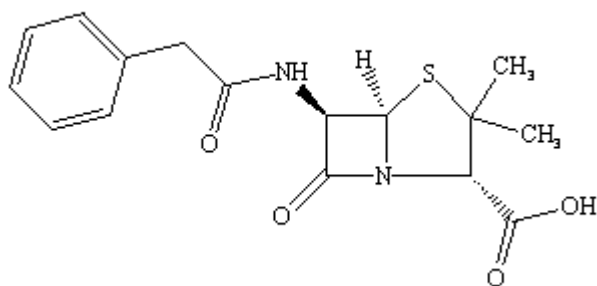


Fig. 8d: Chemical structure of clavulanic acid

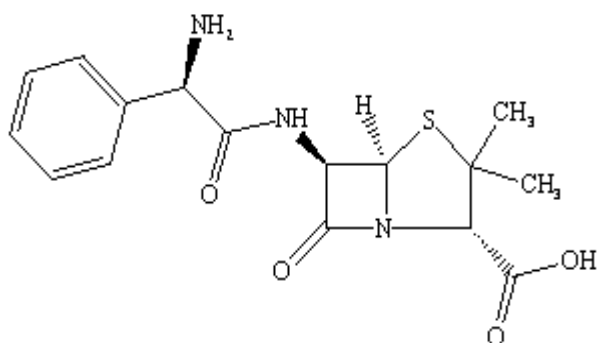
The targets for β -lactam drugs are the penicillin binding proteins (PBP's), so called because they bind radioactive penicillin and can be detected by autoradiography of gels on which bacterial proteins have been separated electrophoretically. The penicillin binding proteins have transpeptidase or carboxypeptidase activity and they act to regulate cell size and shape. They are also involved in septum formation and cell division. Bacteria have several penicillin binding proteins, each with a separate function. Conventionally these are numbered according to size, with PBP 1 as the largest protein. The PBP 1 of one bacterium does not necessarily have the

same function as the PBP 1 of a different organism. The β -lactam antibiotics (Fig. 9; i to iv) may bind preferentially to different penicillin binding proteins, and at sublethal concentrations may cause alterations in cell morphology. For example, mecillinam binds preferentially to *Escherichia coli* PBP 2 and causes formation of spherical cells, whereas cephalexin causes *Escherichia coli* to grow as filaments as a result of its preferential binding to PBP 3. This indicates that PBP 2 in *Escherichia coli* is involved in cell elongation whereas PBP 3 has a role in the cell division of this bacterium.

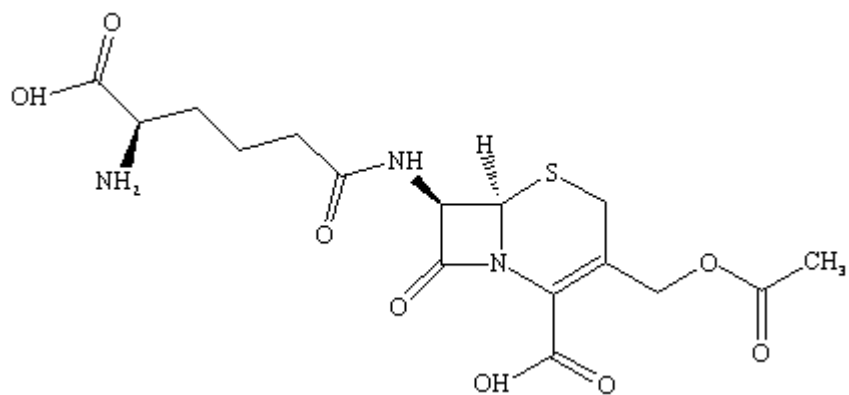
The β -lactam antibiotics also stimulate the activity of autolysins. These are enzymes that are responsible for the natural turnover of cell wall polymers to permit growth of the cells. Under normal conditions, these enzymes produce controlled weak points within the peptidoglycan structure to allow for expansion of the cell wall structure. This activity is stimulated by β -lactams, causing a breakdown of peptidoglycan and leading to osmotic fragility of the cell and ultimately to lysis of the cell.



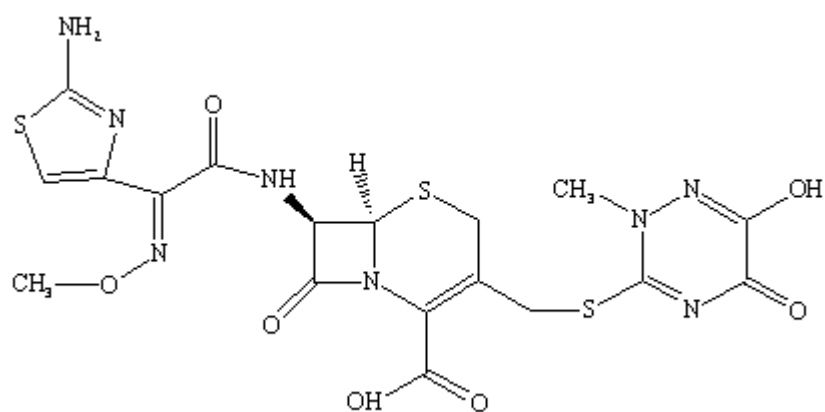
i. Benzyl penicillin



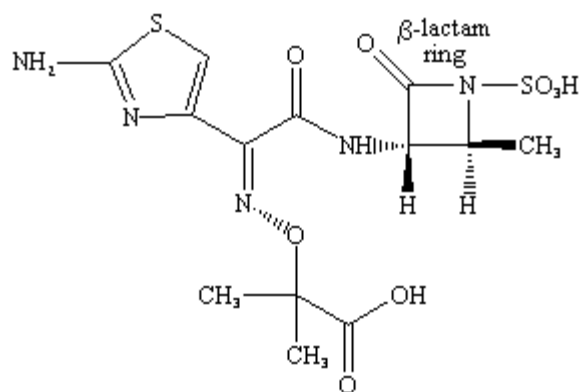
ii. Ampicillin



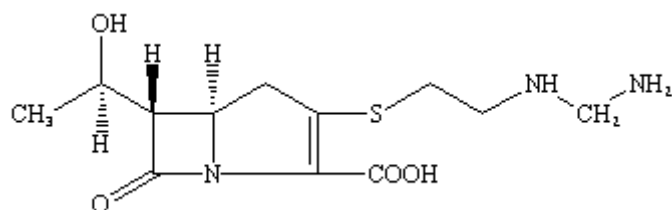
iii. **Cephalosporin C**



iv. **Ceftriaxone**



v. **Aztreonam**



vi Imipenem

Fig. 9: Chemical structures of some β -lactam antibiotics

Vancomycin

The molecule of vancomycin is relatively large (Fig. 10). The drug prevents peptidoglycan subunits from being added to the growing cell wall polymer. This is accomplished by vancomycin binding to the D-alanyl D-alanine residue of the lipid-bound precursor. Its primary activity is against Gram-positive bacteria and thus it is particularly useful in the treatment of serious staphylococcal infections. It is given either intramuscularly or intravenously since it is not absorbed from the gut. It is also used for the treatment of pseudo-membranous colitis caused by *Clostridium difficile* when administered orally.

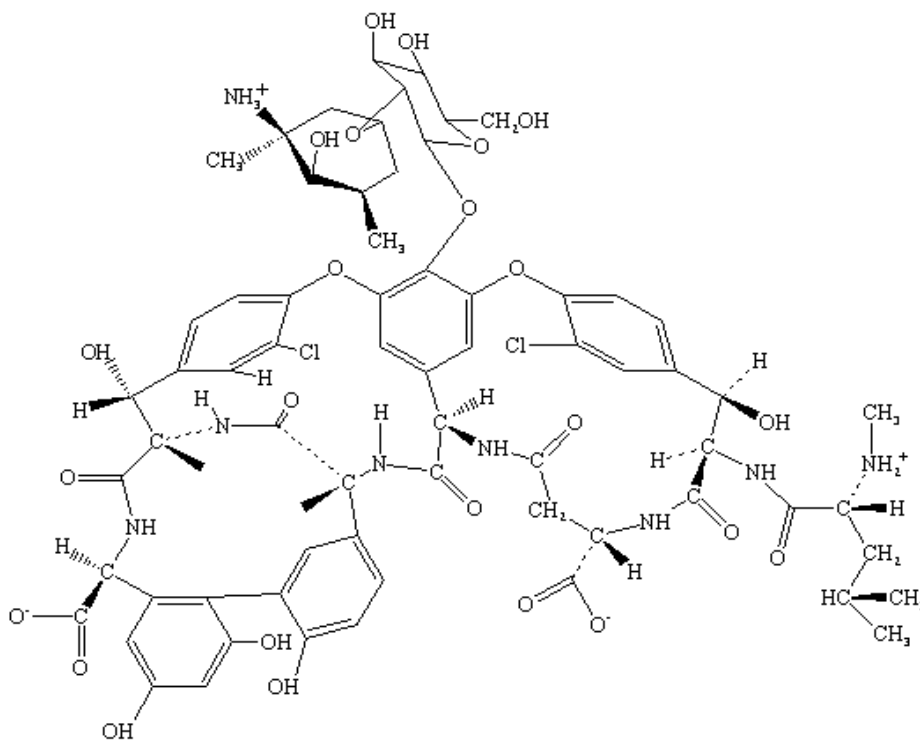


Fig.10: Chemical structure of vancomycin

Fosfomycin

The condensation reaction between UDP-N-acetyl glucosamine and phosphoenol pyruvate in the early stages of peptidoglycan synthesis is the target for fosfomycin (Fig. 11). There is rapid selection of resistance to fosfomycin, rendering it unsuitable for most clinical purposes.

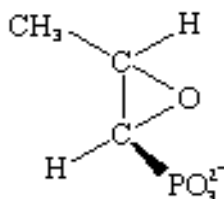


Fig. 11: Chemical structure of fosfomycin

Cycloserine

The simple, cyclic molecule cycloserine is an analogue of alanine (Fig. 12) that interferes with two steps in peptidoglycan synthesis. It is a competitive inhibitor of the racemase that converts L-alanine to D-alanine and it also prevents the action of the D-alanyl D-alanine synthetase. The stable ring structure of cycloserine holds the molecule in a sterically favourable position, permitting preferential binding of this compound both to the racemase and to the synthetase, rather than their natural substrates. This results in competitive inhibition of these enzymes. Cycloserine is a neurotoxin and is not used clinically except for the treatment of life-threatening infections where alternative therapies have failed.

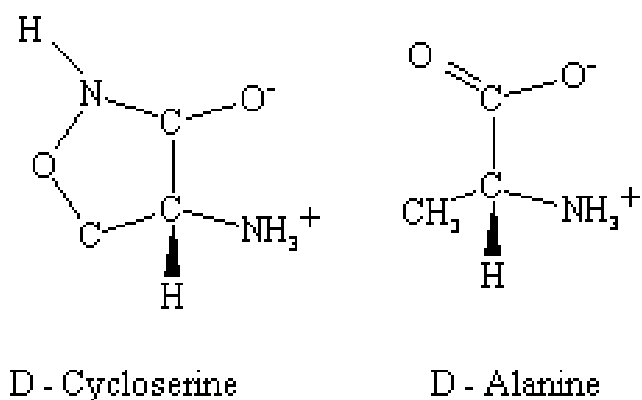


Fig. 12: Chemical structure of cycloserine and its analogue, D-alanine

Bacitracin

The polypeptide antibiotic **bacitracin** is too toxic for human clinical use (Fig. 13). It is, however, widely used in diagnostic laboratories to distinguish bacitracin-sensitive *Streptococcus pyogenes* from other β -hemolytic streptococci. Its activity depends upon its ability to bind to the undecaprenyl pyrophosphate lipid carrier that transports the peptidoglycan monomers across the

bacterial membrane. This blocks the dephosphorylation of the carrier, which, in turn, obstructs regeneration of the undecaprenyl phosphate and thus prevents recycling of the transport mechanism. Bacitracin also interferes with sterol synthesis in mammalian cells by binding to pyrophosphate intermediates thus leading to cyto-toxicity in human.

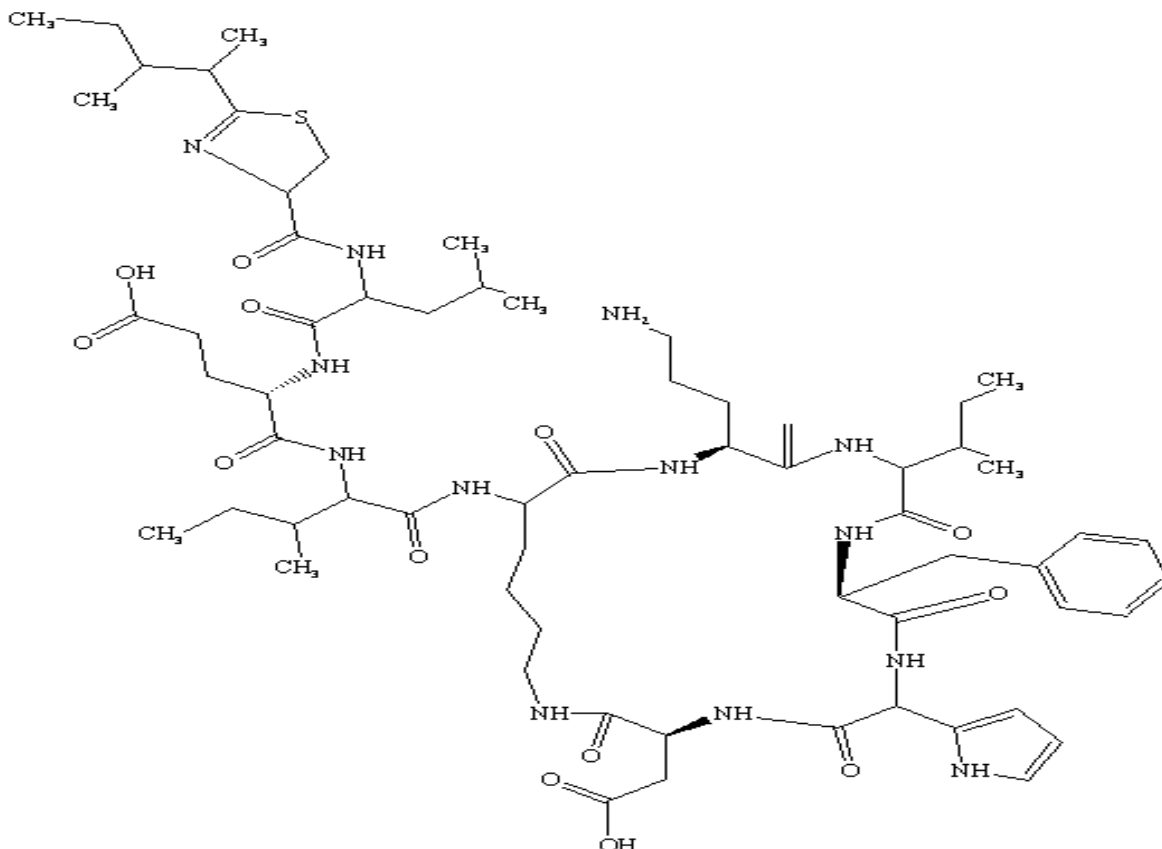


Fig. 13: Chemical structure of bacitracin

Clavulanic acid is a beta-lactamase inhibitor. It is derived from the *Streptomyces clavuligerus* microorganisms from which clavulanic acid is derived. Clavulanic acid is biosynthetically generated from the amino acid arginine. Clavulanic acid is combined with penicillin group antibiotics to overcome certain types of antibiotic resistance. Specifically, it is used to overcome resistance in bacteria that secrete beta-lactamase enzymes, which otherwise inactivate most penicillins. Most commonly, the potassium salt potassium clavulanate is combined with amoxicillin (co-amoxiclav) or ticarcillin.

Other inhibitors of β -lactamase are also combined with β -lactam antibiotics to treat the β -lactam resistant organisms such as Tazobactam. Tazobactam is added to the extended spectrum beta-lactam antibiotic piperacillin to produce Tazocin® or Zosyn®. It broadens the spectrum of piperacillin by making it effective against organisms that express beta-lactamase and would normally degrade piperacillin.

ii) Antibacterial agents affecting bacterial cell membrane function

Antibiotics including the polymixins (Fig. 14) and gramicidin (Fig. 15) act by interfering with the functioning of the bacterial cell membrane by increasing its permeability. Gramicidin is one of a family of cyclic decapeptides active against Gram-positive bacteria. Polymixins have a smaller peptide ring attached to a peptide chain ending with a branched fatty acid. They act specifically against Gram-negative bacteria, although chemically modified derivatives do have a broader spectrum of activity. These antibiotics are toxic to humans and are rarely used in clinical practice now.

dab = l-diaminobutyric acid

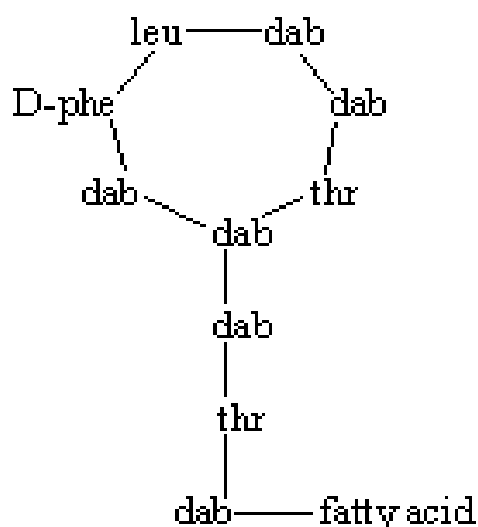


Fig. 14: Chemical structure of a polymixin (for polymixin B, the fatty acid is 6-methyloctanoic acid)

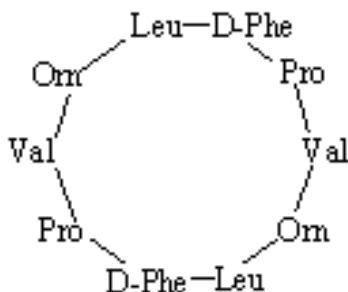


Fig. 15: Chemical structure of gramicidin

The broad-spectrum drug **metronidazole**, an anti-anaerobic and anti-protozoan agent, acts primarily by inhibiting DNA gyrase. It probably also affects bacterial cell membrane as a secondary target.

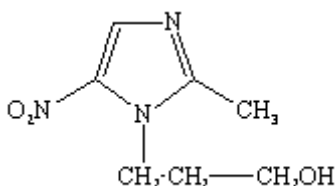


Fig. 16: Chemical structure of metronidazole

iii) Antibacterial agents that inhibit nucleic acid metabolism

Nucleic acid metabolism may be interrupted at many steps. Antibacterial agents show selective toxicity either because humans lack the metabolic processes that act as targets, or because the bacterial targets are much more susceptible to particular chemicals than their eukaryotic counterparts.

Sulphonamides and trimethoprim

Humans are unable to make folic acid, a precursor of purine synthesis. We require an exogenous supply of this metabolite obtained from our diet. Many bacteria are, however, able to generate folic acid from *para*-amino benzoic acid (PABA) and this pathway provides a target for synthetic antimicrobial agents like the sulphonamides (Fig. 17b) and trimethoprim (Fig. 17c). Sulphonamides act by inhibition of dihydropteroate synthetase because it acts as a structural analogue of the normal substrate, PABA (Fig 17a). Trimethoprim inhibits dihydrofolate reductase, the next step in the folic acid biosynthetic pathway. Trimethoprim was first introduced to be used in combination with sulphonamides to potentiate their activity. Studies of their combination *in vitro* show that the combination is synergistic. This means that the combined activity of the drugs is more effective than the additive action of the individual components. The synergism observed *in vitro*, however depends upon maintaining a critical ratio of the two antimicrobials. Because of pharmacological constraints, this cannot be achieved in the body, raising doubts about the synergism *in vivo*. Furthermore, using two agents for chemotherapy significantly increases the risk of the patient developing an adverse reaction to the treatment. Such arguments led to the introduction and successful use of trimethoprim as a single agent.

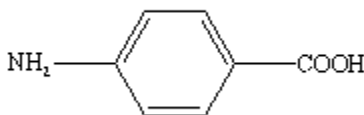


Fig. 17a: Chemical structure of p-aminobenzoic acid (PABA)

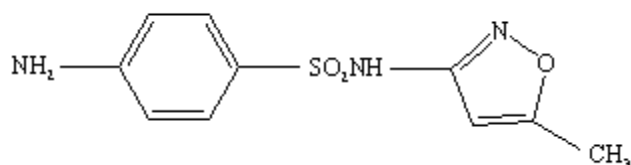


Fig. 17b: Chemical structure of sulphamethoxazole, a sulphonamide

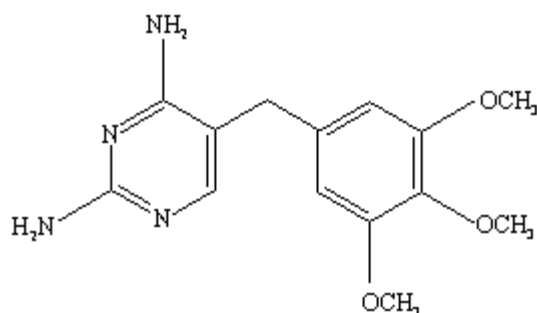


Fig. 17c: Chemical structure of trimethoprim

Quinolones

Bacterial DNA exists in a supercoiled form and the enzyme DNA gyrase, a topoisomerase, is responsible for introducing negative supercoils into the structure. Quinolone antibacterial drugs such as nalidixic acid (Fig. 18a), norfloxacin, ofloxacin and ciprofloxacin (Fig. 18b) act by inhibiting the activity of the bacterial DNA gyrase, preventing the normal functioning of DNA. Humans do possess DNA gyrase but it is structurally distinct from the bacterial enzyme and remains unaffected by the activity of quinolones. These are broad-spectrum agents that rapidly kill bacteria and are well absorbed after oral administration. Overuse of these drugs in certain situations is selecting quinolone resistant mutants and these have threatened widespread use of such compounds.

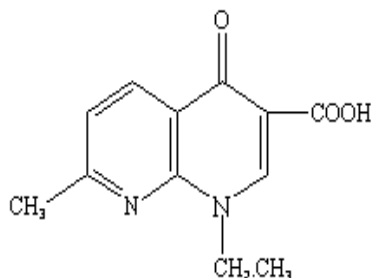


Fig. 18a: Chemical structure of nalidixic acid

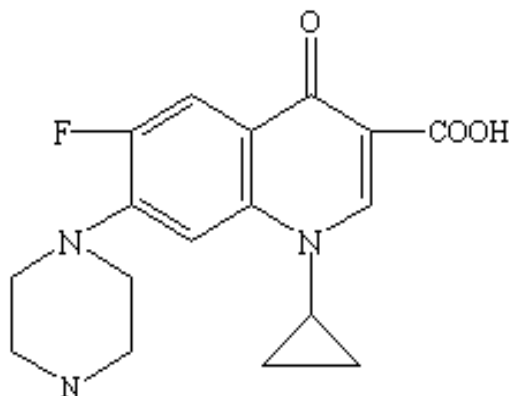


Fig. 18b: Chemical structure of ciprofloxacin

Metronidazole

The broad-spectrum antibiotic **metronidazole**, a very important anti-anaerobic and anti-protozoan agent, probably has a similar primary mode of action as the quinolones, although it also affects cell membrane function.

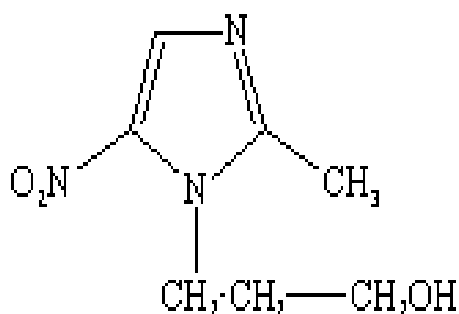


Fig. 19: Chemical structure of metronidazole

iv) Antibacterial agents that inhibit RNA metabolism

The bacterial DNA-dependent RNA polymerase is inhibited by rifampicin (Fig. 20a) but this drug has little effect on eukaryotic cells. It is active against the mitochondrial RNA polymerase but its penetration into mitochondria is so poor that it displays very little activity in intact eukaryotic cells. The action of rifampicin prevents production of messenger RNA and thus ultimately stops protein synthesis. Clinically, rifampicin is used in treating tuberculosis and for prophylaxis against meningococcal meningitis. In such cases, it is offered to close contacts of people with the disease. The synthetic antibacterial nitrofurantoin compounds (Fig. 20b) also act by preventing messenger RNA production.

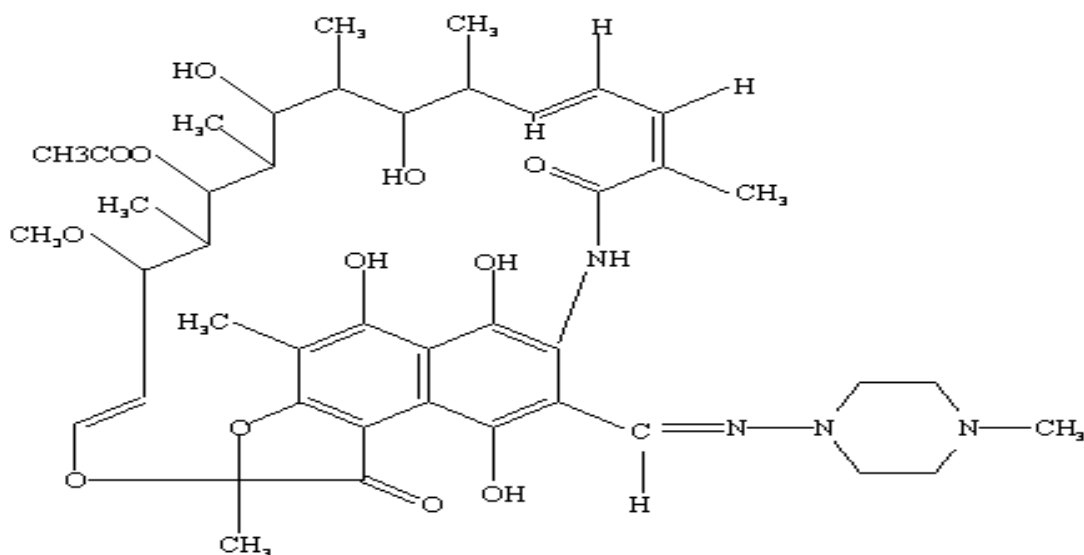


Fig. 20a: Chemical structure of rifampicin

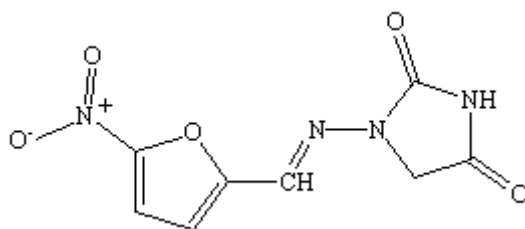


Fig. 20b: Chemical structure of nitrofurantoin

v) Antibacterial agents that inhibit protein synthesis

Aminoglycosides

The aminoglycosides are a clinically important group of bactericidal antibiotics that have a broad-spectrum of activity. The family includes streptomycin (Fig. 21), gentamicin, tobramycin, kanamycin, amikacin and netilmicin. The aminocyclitol such as spectinomycin are closely related and have a similar mode of action. Aminoglycosides have a variety of effects within the bacterial cell but principally these inhibit protein synthesis by binding to the 30S ribosomal subunit to prevent the formation of an initiation complex with messenger RNA. They also cause misreading of the messenger RNA message, leading to the production of nonsense peptides. Another important function of the aminoglycosides is that they increase membrane leakage. Antibiotics such as gentamicin and kanamycin exist as mixtures of several closely related structural compounds; while netilmicin and amikacin have a single molecular structure.



Tetracyclines

The tetracyclines (Fig. 22) are a family of broad-spectrum antibiotics possessing a four-ring structure. They inhibit binding of the aminoacyl tRNA to the 30S ribosomal subunit in bacteria. The action is bacteriostatic and can be reversed upon removal of the drug. The clinical use of tetracyclines is generally confined to adults. This is because tetracyclines affect bone development and can cause staining of teeth in children. Tigecycline is a new tetracycline that is active against meticillin-resistant *Staphylococcus aureus*.





The chloramphenicol (Fig. 24) is another broad-spectrum bacteriostatic agent that is toxic to humans. This drug may cause aplastic anemia and thus its use is confined to life-threatening infections where no alternative therapy is available. It acts by binding to the 50S ribosomal subunit and blocking the formation of the peptide bond by inhibiting peptidyl transferase activity. It is a potent inhibitor of mitochondrial protein synthesis in eukaryotic cells.



The macrolides are a group of antibiotics that have a large lactone ring structure. These may be 14- or 16-membered rings (Fig. 25 and 26). The most widely used macrolides are erythromycin (Fig. 25a) and clarithromycin. These relatively non-toxic antibiotics are most active against Gram-positive bacteria. Erythromycin is, however, the treatment of choice for Legionnaire's disease caused by the Gram-negative bacillus *Legionella pneumophila* and it is also active against *Haemophilus influenzae*, another Gram-negative bacillus. Erythromycin binds to the 50S

ribosomal subunit and inhibits either peptidyl transferase activity or translocation of the growing peptide. Newer macrolides include azithromycin (Fig. 25b) and clarithromycin. These have the same activity as erythromycin but they have better pharmacological properties.

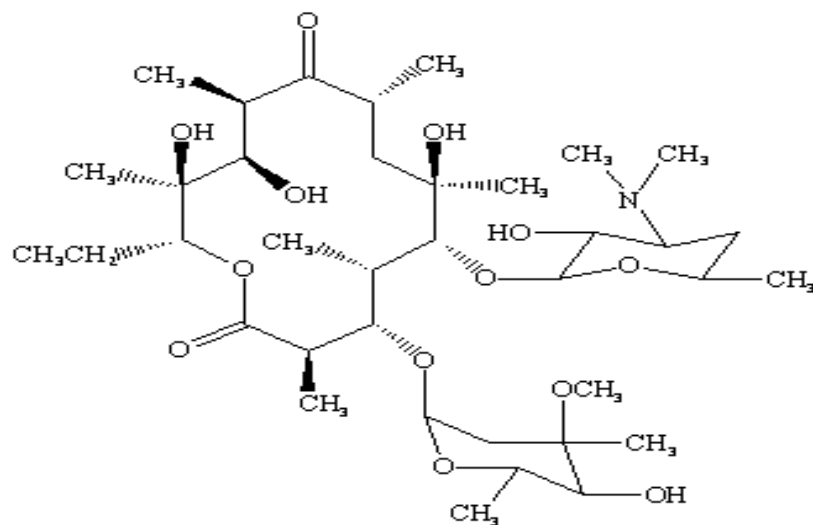


Fig. 25a: Structure of erythromycin

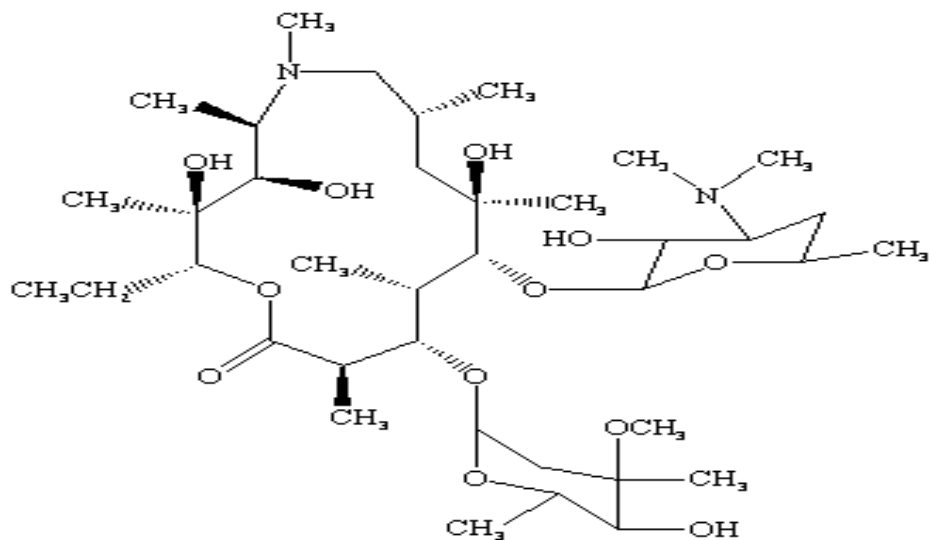


Fig. 25b: Structure of azithromycin

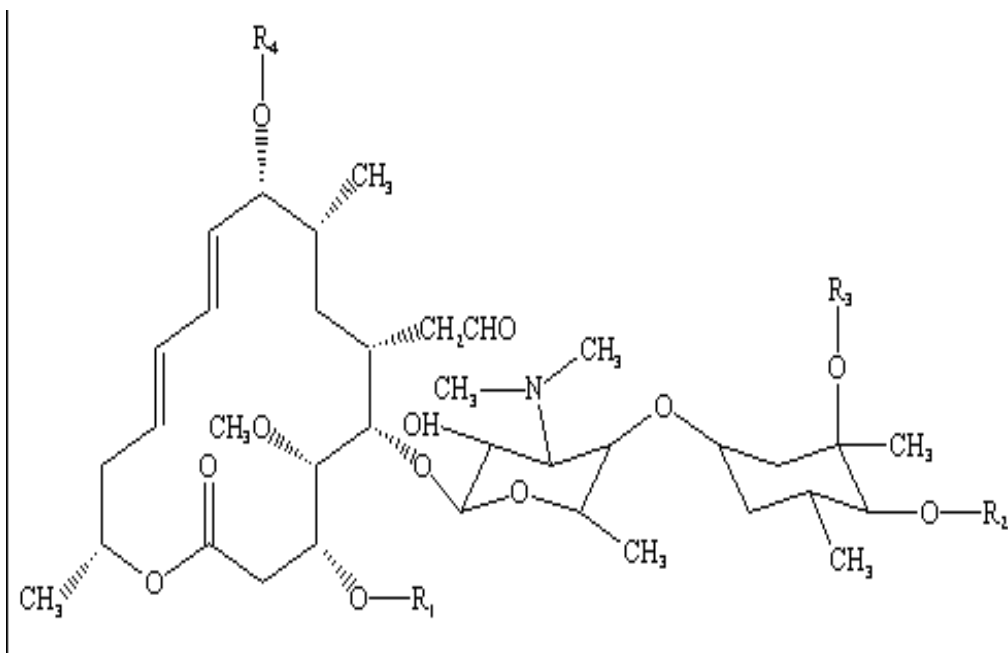


Fig. 26: Chemical structure of a macrolide with a 16-membered ring

The lincosamide antibiotic **lincomycin** (Fig. 27a) and its semi-synthetic derivative **clindamycin** (Fig. 27b) have a similar mode of action.

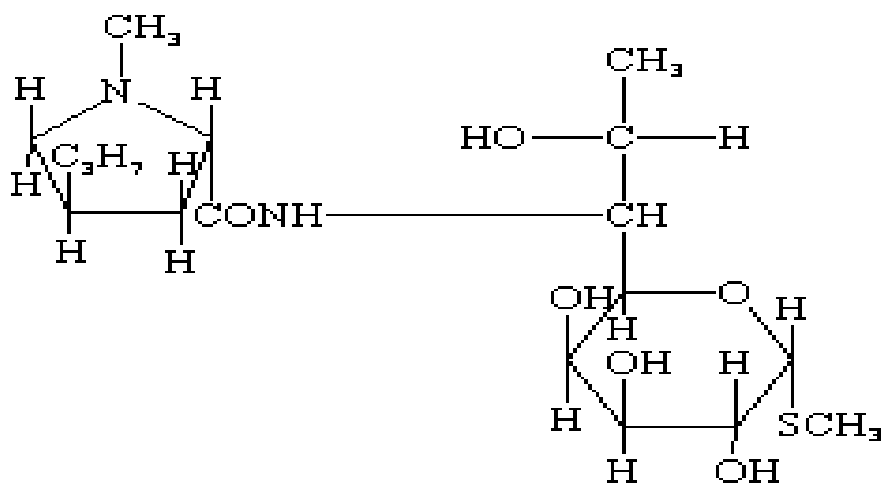


Fig. 27a: Chemical structure of lincomycin

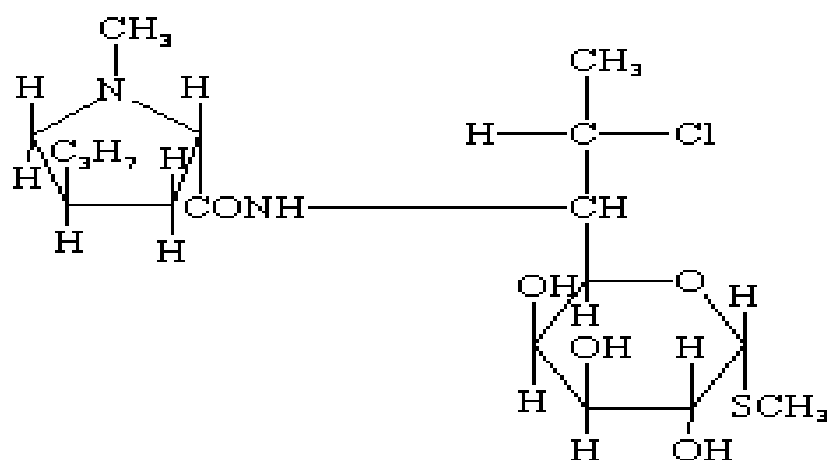


Fig. 27b: Chemical structure of clindamycin

Fusidic acid

A steroid antibiotic fusidic acid (Fig. 28) is used to treat Gram-positive infections. It acts by preventing translocation of peptidyl tRNA. Fusidic acid is usually administered in combination with another antibiotic to avoid the selection of resistant bacterial strains. To survive, the fusidic acid resistant strains must also become resistant to the antibiotic given in combination.

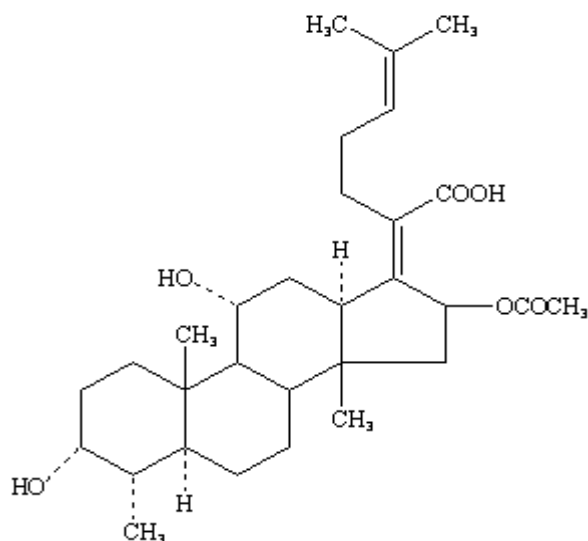


Fig. 28: Chemical structure of fusidic acid

Streptogramins

The streptogramins fall into two groups, A (Fig. 29a) and B (Fig. 29b). Streptogramins belonging to Group A have a large non-peptide ring, which is polyunsaturated. Streptogramins related to streptogramin B are cyclic peptides. They differ in their modes of action although both inhibit bacterial protein synthesis. Group A streptogramins distort the ribosome to prevent binding of the t-RNA; Group B streptogramins are thought to block translocation of the growing peptide.

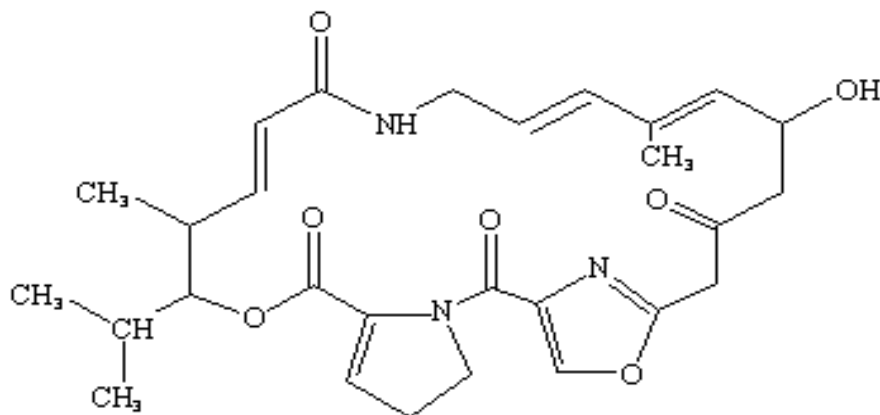


Fig. 29a: Chemical structure of streptogramin A

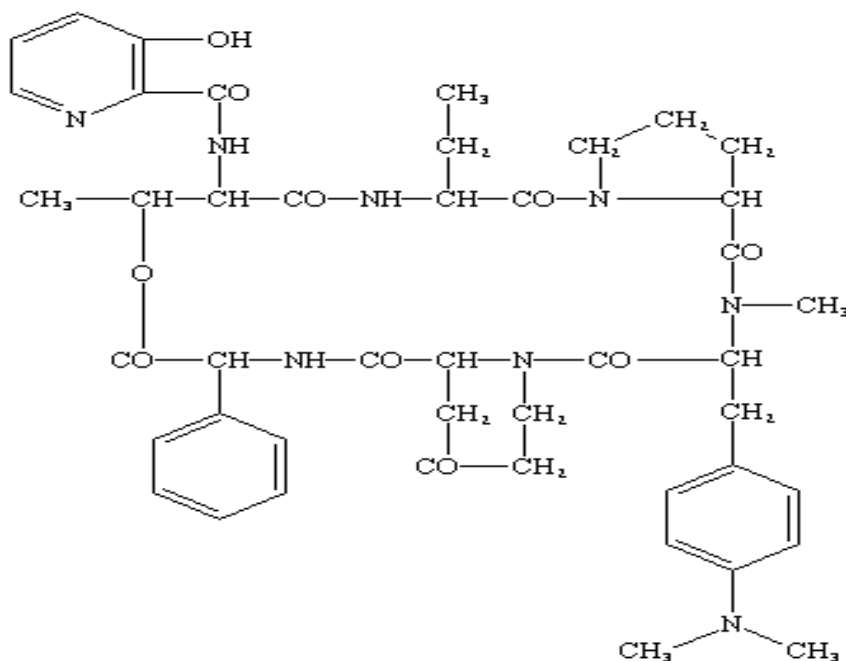


Fig. 29b: Chemical structure of streptogramin B

A combination of dalfopristin (Fig. 29c) and quinupristin (Fig. 29b) has recently been introduced for use in treatment of methicillin-resistant *Staphylococcus aureus*. Dalfopristin is a Type A

streptogramin (Fig. 33c) and quinupristin is a Type B streptogramin (Fig. 33d). In combination, these drugs show a synergistic effect.

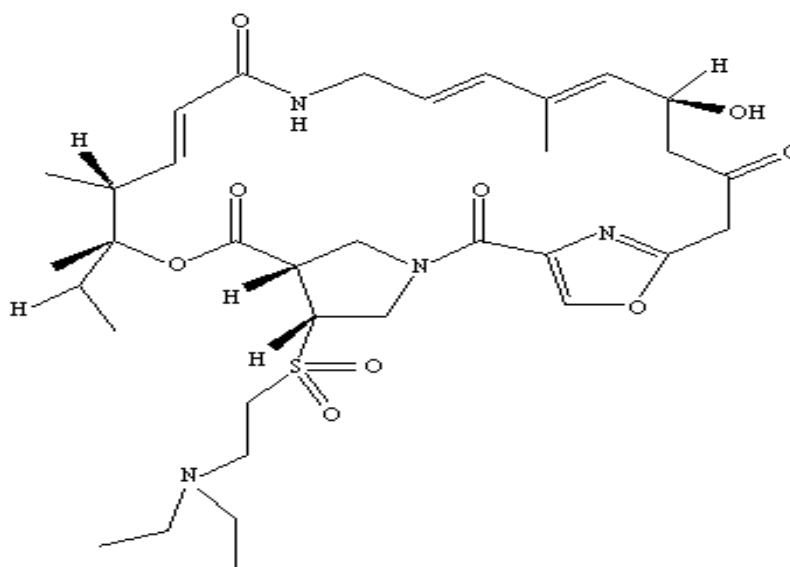


Fig. 29c: Chemical structure of dalfopristin

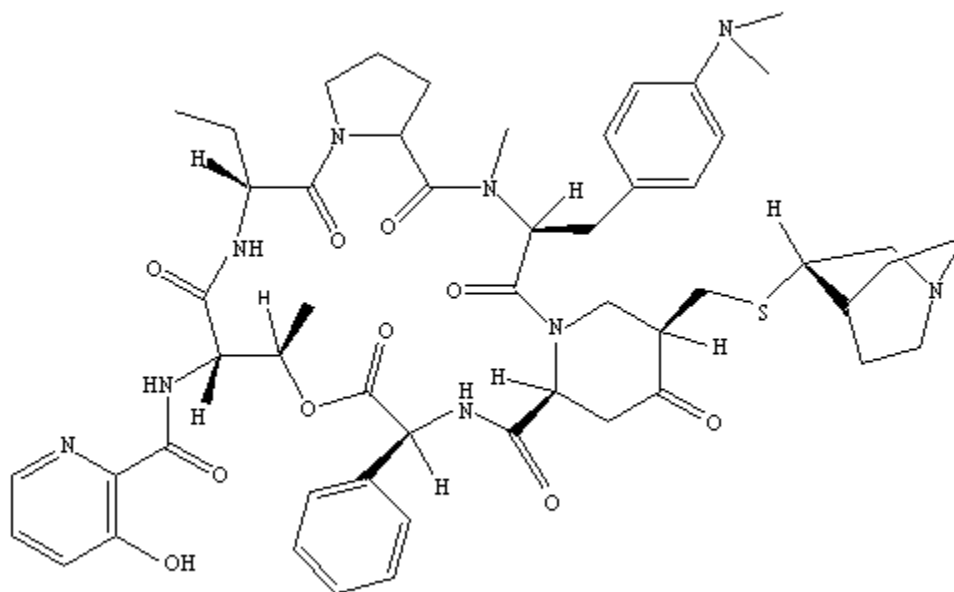


Fig. 29d: Chemical structure of quintupristin

Mupirocin

Mupirocin (Fig. 30) is an analogue of iso-leucine. It inhibits the iso-leucyl-transfer RNA synthetase, thereby preventing the incorporation of iso-leucine into growing polypeptide chains. It is not toxic to humans but can only be used topically for skin infections. Humans rapidly metabolize the drug to an inactive form and thus in systemic therapy it is destroyed before it can be effective.

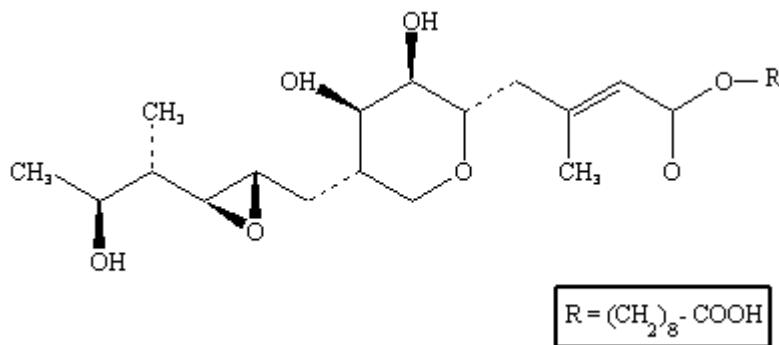


Fig. 30: Chemical structure of mupirocin

Linezolid

Linezolid is a new class of bacterial protein synthesis inhibitor, the oxazolidinone antibiotic (Fig. 31). It does so (Fig. 35) by interfering with the interaction between mRNA and the two ribosomal subunits necessary for the initiation of translation of the messenger RNA into the nascent peptide chain. It is active against Gram-positive cocci, including methicillin-resistant *Staphylococcus aureus* and vancomycin resistant enterococci.

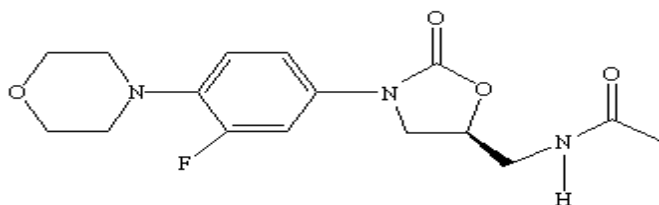


Fig. 31: Chemical structure of linezolid

vi) Anti-mycobacterial drugs

Mycobacteria are unusual bacteria that contain high amount of waxy material in their cell wall. This makes it difficult to stain these bacteria using conventional staining techniques. It also makes penetration of many antimicrobial drugs difficult. As mycobacteria are very slow growing, a protracted anti-mycobacterial therapy is required to treat the patients. Unlike many pathogens, resistance to antimicrobials in mycobacteria typically results from point mutations in

the bacterial chromosome, resulting in changes in the antibiotic target that render it no longer susceptible to the drug. Hence, combinations of drugs are used to treat mycobacterial infections such as leprosy and tuberculosis. Anti mycobacterial drugs are frequently associated with hepato-toxicity. To overcome the toxic side effects of these drugs and to overcome the emergence of resistant strains, treatment is currently recommended as Daily Observed Therapy or DOT.

First discovered by Albert Schatz, working in Selman Walkman's laboratory, streptomycin (a protein synthesis inhibitor) was the first drug used successfully to treat tuberculosis. Rifampicin is also used as an anti-mycobacterial drug that interferes with the DNA-dependent RNA polymerase of bacterial cells (Fig. 32). The action of rifampicin prevents production of messenger RNA and thus ultimately stops protein synthesis. The anti mycobacterial drug isoniazid inhibits the formation of very long chain fatty acids such as those found in the cell walls of mycobacteria. Isoniazid is used in the treatment of tuberculosis and other mycobacterial infections.

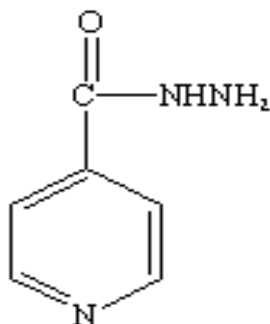


Fig. 32: Chemical structure of isoniazid

Ethambutol is a front-line anti- mycobacterial drug that inhibits cell wall synthesis. However, its mode of action is not well understood (Fig. 33). Pyrazinamide is another anti mycobacterial drug that inhibits mycobacterial metabolism (Fig. 34). Its mode of action also remains to be elucidated fully.

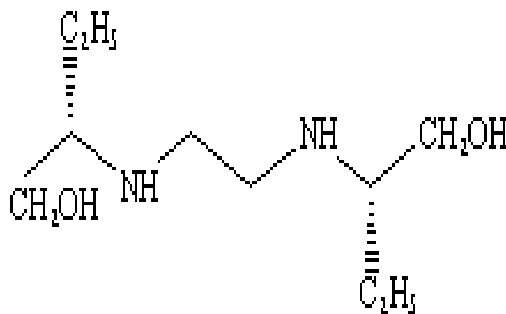


Fig. 33: Chemical structure of ethambutol

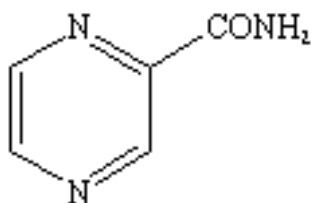


Fig. 34: Chemical structure of pyrazinamide

vii) Antifungal agents

Fungal infections caused by eucaryotic organisms are generally more difficult to treat than the bacterial infections. There are relatively few agents that can be used to treat fungal infections. The fungal cell wall may be considered to be a prime target for selectively toxic antifungal agents because of its chitin structure, which is absent from the human cells. No clinically available inhibitor of chitin synthesis analogous to the β -lactams exists at present, even though much effort is being directed towards developing such agents.

Polyene antibiotics

Polyene antibiotics bind to sterols within the fungal membrane, disrupting its integrity. This makes the membrane leaky, leading to a loss of small molecules from the fungal cell. Polyene antibiotics include nystatin (Fig. 35a), used topically for *Candida* infections. Another polyene antibiotic is amphotericin B (Fig. 35b).

The antifungal drug amphotericin B is administered parenterally and is widely used to treat systemic mycoses. It is most often given intravenously in a bile salt suspension and diluted with 5% dextrose. It penetrates poorly into cerebrospinal fluid and when used to treat meningitis it may be delivered directly into the brain ventricles. Amphotericin B is a very successful and widely used antifungal, which unfortunately possesses toxicity. It can cause unpleasant side effects including chills, fever and a lowering of blood pressure besides kidney damage. The side effects of amphotericin B therapy can mimic the clinical appearance of serious systemic infection, complicating patient's management. The severity of side effects may cause interruption of antifungal infection. Amphotericin B remains the drug of choice for life-threatening fungal infections. It may often be administered together with flucytosine since in combination a lower dose may be used, reducing the risk of therapeutic complications. To avoid toxicity, amphotericin B is now given in the form of liposomal preparation. This is available as Ambisome[®].

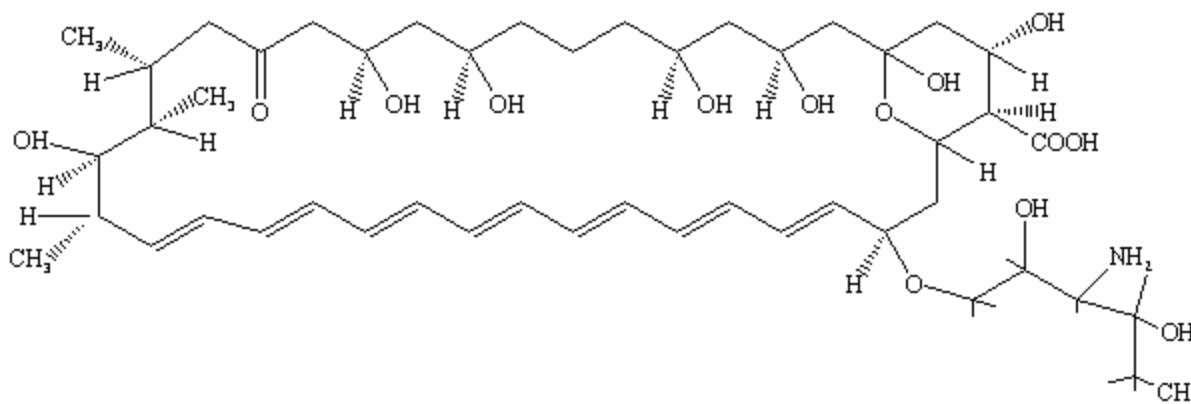


Fig. 35a: Chemical structure of nystatin

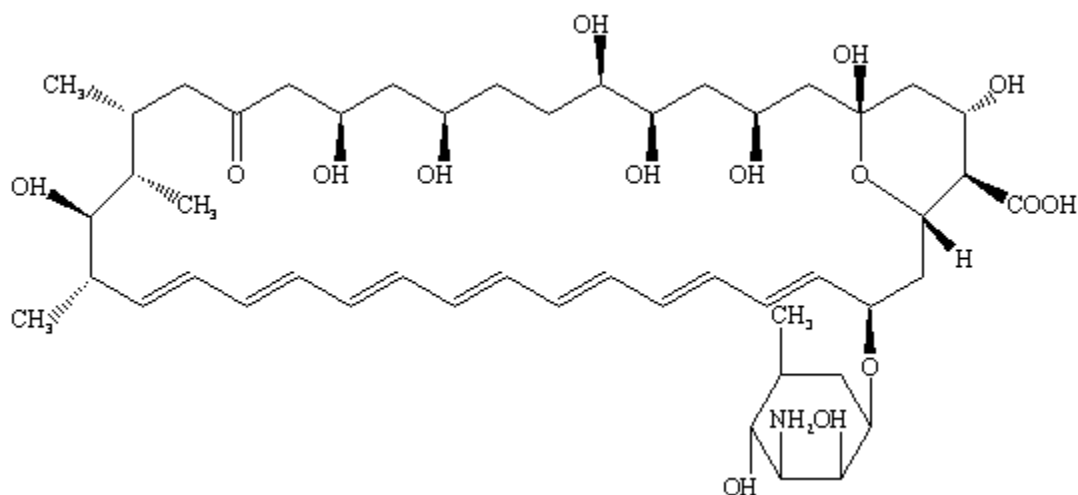


Fig. 35b: Chemical structure of amphotericin B

Imidazoles and triazoles

These are group of broad-spectrum antifungal agents that inhibit synthesis of ergosterol, a component of fungal membranes. These drugs, like the polyene antibiotics, may cause leakage of small molecules out of fungal cells. Drugs in this class include miconazole (Fig. 36a) and ketoconazole (Fig. 36b). They have antifungal activity although there is some variation of activity between the various compounds. Some azoles are also active against Gram-positive bacteria. Clotrimazole is very commonly used topical antifungal agent.

Azoles are known to inhibit the fungal cytochrome P450 enzyme. Some members of the azole group can also affect the human equivalent and are thus toxic to humans. The majority of azoles can only be used topically but some are used to treat systemic infections. Some may be taken orally whereas others must be delivered parenterally. Ketoconazole is administered orally; miconazole is given intravenously. The azoles fluconazole (Fig. 36c) and itraconazole (Fig. 36d) may be delivered either orally or parenterally. These drugs are being evaluated in the treatment

of systemic mycoses and have met with variable success. Fluconazole is excreted through the kidneys. It may accumulate within this tissue, leading to renal damage. Resistance to fluconazole has emerged during therapy. *Candida glabrata* quickly becomes resistant to this drug while *Candida krusei* is not sensitive to this compound.

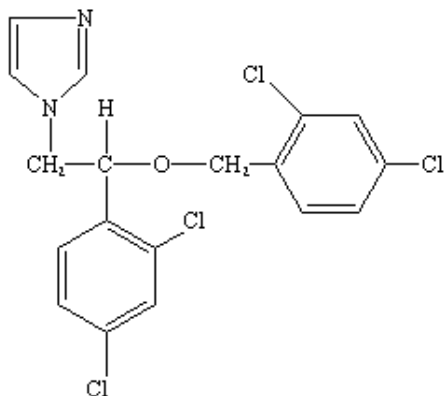


Fig. 36a: Chemical structure of myconazole

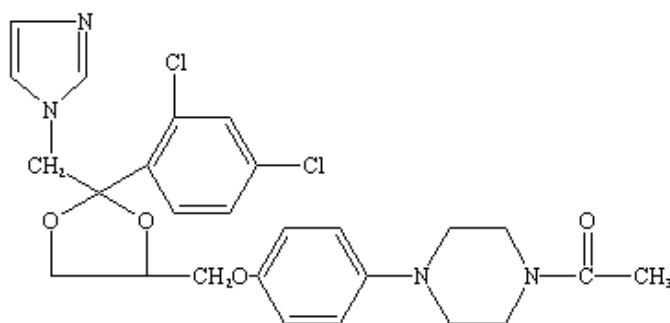


Fig. 36b: Chemical structure of ketoconazole

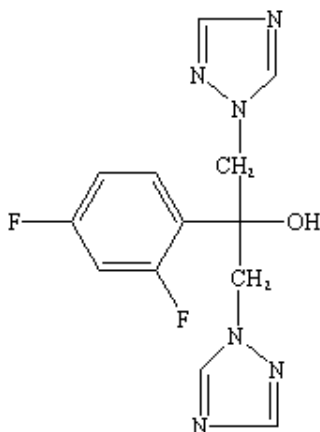


Fig. 36c: Chemical structure of fluconazole

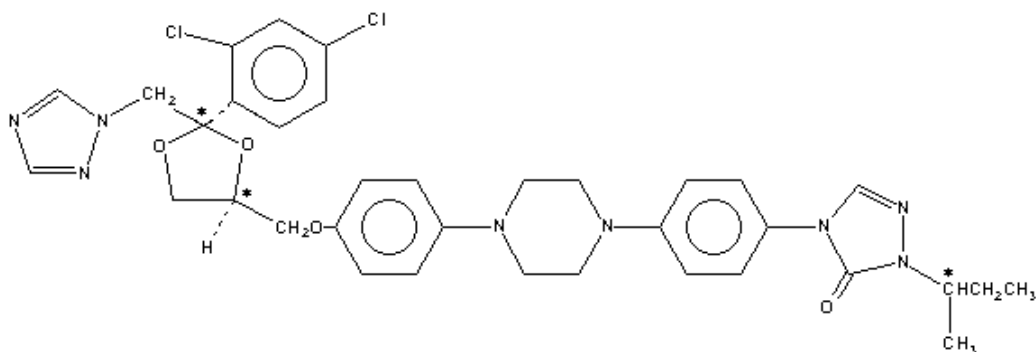


Fig. 36d: Chemical structure of itraconazole

Terbinafine is a synthetic antifungal agent introduced in 1991 and is used to treat skin and nail infections (Fig. 37). It inhibits ergosterol biosynthesis.

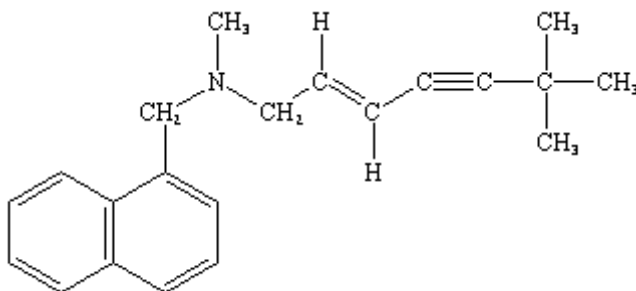


Fig. 37: Chemical structure of terbinafine

Griseofulvin

Griseofulvin is a naturally occurring antifungal antibiotic (Fig. 38). It is obtained from *Penicillium griseofulvum*. It binds to the proteins involved in microtubule formation and prevents separation of chromosomes at mitosis. However, it does not affect human cells. It is used in the treatment of ringworm and other fungal infections of the skin or nails as it tends to accumulate in the layers of cells lying beneath the skin.

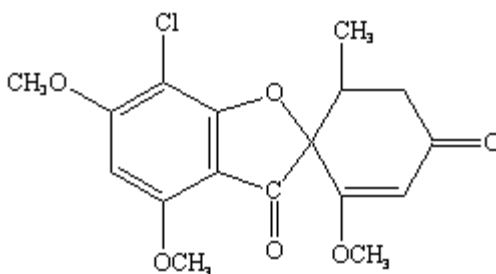


Fig. 38: Chemical structure of griseofulvin

5-Flucytosine

5-Flucytosine, a synthetic pyrimidine interferes with the nucleic acid metabolism in fungi (Fig. 39). Being an analogue of naturally occurring nucleotide, the metabolizing cells convert it to 5-fluorouracil. It is used primarily to treat systemic *Candida* infections. It may be given orally or parenterally. It can be used as a single agent or in combination with other antifungal drugs including amphotericin B. 5-flucytosine achieves good penetration into body fluids, including cerebrospinal fluid. It is excreted through the kidneys and its dosage must be modified in patients with renal problems. It derives its selective toxicity from the inability of human cells to convert it to 5-fluorouracil. Its major drawback is the ease with which resistance develops. Susceptibility testing of fungal isolates is necessary during treatment so that resistant variants may be detected early and alternative therapy may be started.

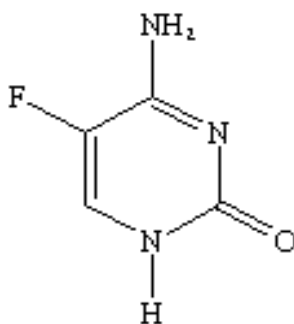


Fig. 39: Chemical structure of 5-flucytosine

viii) Antiviral agents

Viruses are intracellular pathogens, and very few clinically useful agents are currently available to treat virus infections. Development of drugs that are active against viruses is one of the most challenging areas in antimicrobial chemotherapy.

Acyclovir

The purine acyclovir, or acycloguanosine (Fig. 40), inhibits the thymidine kinase of herpes viruses. It is particularly active against herpes simplex virus types 1 and 2, is less active against *Varicella zoster* virus and has significantly reduced activity against cytomegalovirus. It has prevented significant mortality associated with herpes simplex encephalitis and is of value in treating *Varicella zoster* infections in immunocompromised patients. Acyclovir is also used for the treatment of severe cases of genital herpes. Resistance to acyclovir because of mutations in the thymidine kinase gene limits the use of this drug for more trivial conditions. It does not eradicate latent virus but acyclovir does shorten the duration of clinical symptoms. **Gancyclovir** is an analogue of acyclovir that is active against cytomegalovirus as well as other herpes viruses (Fig. 41).

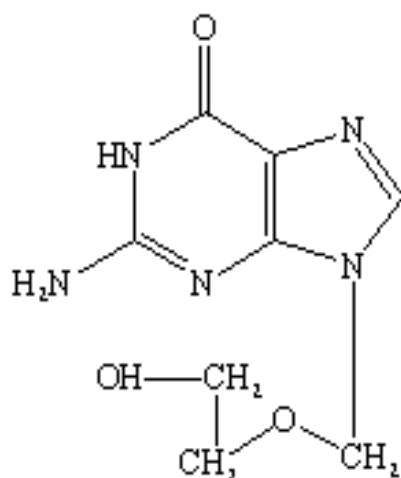


Fig. 40: Chemical structure of acyclovir

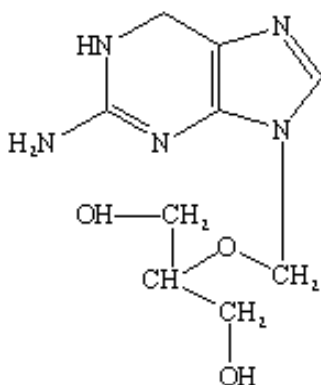


Fig. 41: Chemical structure of ganciclovir

Amantidine

Amantidine is used to treat shingles, particularly in elderly or debilitated patients (Fig. 42). It is also used for the treatment of influenza A virus infections. Its mode of action has not been fully elucidated. It is thought to act by interfering with the uncoating of the virus.

Ribavirin

Ribavirin is a broad-spectrum drug active against both RNA and DNA viruses (Fig. 43). In the Developed World, its principal use is in treating severe respiratory syncytial virus infections in infants. The virus infects the bronchioles, causing bronchiolitis and the drug may be inhaled. Its mode of action is unclear.

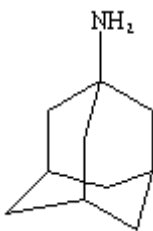


Fig. 42: Chemical structure of amantidine

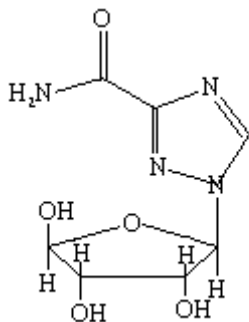


Fig. 43: Chemical structure of ribavirin

Zidovudine

This was formerly called azidothymidine and is also known as AZT (Fig. 44). It is an anti-retrovirus agent that is phosphorylated to form a triphosphate derivative inside infected and uninfected cells. Zidovudine triphosphate is a competitive inhibitor of the retrovirus reverse transcriptase, the enzyme that produces provirus DNA from the virus RNA template. Its principal use is in the management of patients with advanced human immunodeficiency virus disease (AIDS).

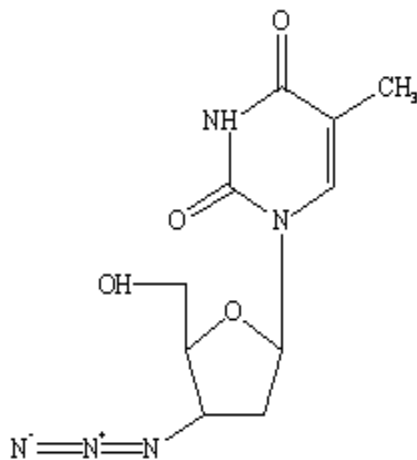


Fig. 44: Chemical structure of zidovudine

Antibiotic resistance in bacteria

Bacteria have evolved many strategies for resisting the action of antibiotics and antibacterial agents. This is particularly true of those bacteria that are antibiotic producers. Bacteria that produce antibiotics do so to gain a selective advantage over other competing microbes in their natural environment. If they were sensitive to their own metabolic products, such a selective advantage would be lost. In many hospital units, exploitation of antibiotics is very intensive and this generates an enormous selective pressure for bacteria to acquire the means by which they may become antibiotic-resistant. Under such circumstances, it is not unusual to find that bacteria exhibit resistance to more than one group of antibiotics. Resistance to a particular agent may be accomplished by more than one resistance mechanism. Bacteria may display antibiotic resistance by one or the other mechanisms (Table 1).

Table 1: Various mechanisms of antibiotic resistance in bacteria

S. No.	Mechanism	Example(s)
1	Lack of target for the antibiotic	<i>Chlamydiae</i> do not have peptidoglycan and are not susceptible to the action of penicillins.
2	Antibiotic target may be inaccessible	Peptidoglycan in Gram-negative bacteria is inaccessible to penicillins that cannot penetrate the Gram-negative outer membrane. Efflux pumps can actively pump out antibiotics from cells. Gram-negative bacteria resist the activity of tetracyclines by this important mechanism.
3	Antibiotic target may be modified to prevent the action of the drug	Trimethoprim resistance is manifest by alterations in the dihydrofolate reductase (DHFR) target enzyme; quinolone resistance is affected by point mutations in the DNA gyrase, which prevent binding of the drug to its target.
4	Antibiotic may be chemically modified or destroyed	Range of β -lactamase and the various aminoglycoside-modifying enzymes. Chloramphenicol resistance is most often manifest by acetylation by the chloramphenicol acetyl transferase enzyme.
5	Bacteria may elaborate alternative pathways, avoiding the drug target	Meticillin resistance in meticillin-resistant <i>Staphylococcus aureus</i> results from the production of an additional penicillin binding protein: PBP2', which is not susceptible to inhibition by penicillins.
6	Lack of peptidoglycan	Rickettsias and chlamydia lack peptidoglycan and hence resistant to the action of cell wall inhibitors such as the penicillins and cephalosporins.
7	Blocking of entry of drug (Presence of outer membrane in Gram negative bacteria)	Resistant to antibiotics like benzyl penicillin because such drugs cannot penetrate the outer membrane and so cannot reach their target.
	Active efflux of drugs by micro-organisms	Gram-negative bacteria may resist the activity of tetracyclines through an energy-dependent active efflux of the drug.

Alterations to the side chain attached to the penicillin nucleus may overcome the problem of membrane penetration and semi-synthetic penicillins such as ampicillin have a broad-spectrum of activity, encompassing both Gram-positive and Gram-negative bacteria. Modification of the antibiotic target is often seen in laboratory generated drug-resistant mutants. For example, bacteria resistant to trimethoprim produce an alternative dihydrofolate reductase (DHFR). Resistance to the quinolone antimicrobials results from point mutations in the gene encoding DNA gyrase. Aminoglycoside resistance may result from modifications of the ribosome structure. Indeed, in the laboratory, ribosomes may be further altered so that they only function in the presence of aminoglycosides. The drug acts to stabilize the functional ribosome in aminoglycoside-dependent bacteria. Many clinically important bacteria produce enzymes that are capable of chemically modifying or destroying antibiotics. Chloramphenicol may be acetylated by the action of chloramphenicol acetyltransferases. Aminoglycosides may be acetylated by aminoglycoside acetyltransferases, phosphorylated by aminoglycoside phosphotransferases or conjugated with nucleotides. Such modifications render the antibiotic inactive.

Antibiotics may also be enzymatically degraded to an inactive form. β -lactamases can hydrolyze the β -lactam bonds. Some β -lactamases have a preferential activity against penicillins and these are referred to as penicillinases. Cephalosporinases are more active against cephalosporins. Recently broad-spectrum β -lactamases have evolved that have activity against both penicillins and cephalosporins. There are families of such enzymes that have arisen as the result of point mutations accumulating in the genes that code for penicillinases. Extended-spectrum β -lactamases (ESBLs) are enzymes that mediate resistance to extended-spectrum (third generation) cephalosporins (e.g. ceftazidime, cefotaxime, and ceftriaxone) and monobactams (e.g. aztreonam) but do not affect cephamycins (e.g., cefoxitin and cefotetan) or carbapenems (e.g., meropenem or imipenem). The presence of an ESBL-producing organism in a clinical infection can result in treatment failure if one of the above classes of drugs is used. ESBLs can be difficult to detect because they have different levels of activity against various cephalosporins. Thus, the choice of which antimicrobial agents to test is critical. If an ESBL is detected, all penicillins, cephalosporins, and aztreonam should be reported as resistant, even if in vitro test results indicate susceptibility. The National Committee for Clinical Laboratory Standards (NCCLS) has developed broth microdilution and disk diffusion screening tests for phenotypic confirmation of potential ESBL-producing isolates using selected antimicrobial agents.

Many of these new enzymes are encoded by self-transmissible plasmids and these new resistance determinants can spread with great ease. Not all β -lactamase activity is associated with bacterial cells. Human kidney cells produce an analogous enzyme that, although it does not readily attack penicillins and cephalosporins, rapidly destroys carbapenems such as imipenem. Because of this, imipenem is administered together with cilastatin, an inhibitor of the human kidney enzyme. This delays the breakdown of imipenem sufficiently to permit it to be active in treating bacterial infection.

Staphylococci have been associated with the production of β -lactamases for many years. Early in the history of the development of semi-synthetic penicillins, compounds were manufactured that were able to resist the activity of staphylococcal penicillinase. These drugs had side-chains that prevented the staphylococcal β -lactamase from binding to the antibiotic and hydrolyzing it. Meticillin, penicillin that is stable in the presence of staphylococcal β -lactamase, was introduced

into clinical practice during the 1960's. Until recently, meticillin was known as "methicillin". Shortly after the introduction of meticillin into medical practice, resistant strains of *Staphylococcus aureus* were isolated from hospital units where the drug was in regular use. Meticillin-resistance is greater at 30°C than at 37°C. Resistance is due to the temperature-sensitive production of an extra penicillin binding protein, PBP 2', that is not susceptible to inhibition by meticillin. Meticillin-resistant *Staphylococcus aureus* (MRSA) also produce a β -lactamase and they are generally resistant to a very wide range of antimicrobials. Infections caused by meticillin-resistant *Staphylococcus aureus* can thus be difficult to treat. In some cases the only drugs available to effectively treat infections caused by meticillin-resistant *Staphylococcus aureus* are the glycopeptides such as vancomycin. Bacterial resistance to this agent was unknown until recently. Vancomycin resistance first appeared in enterococci. These bacteria are resistant to all currently available standard antimicrobial therapies. In an experiment of dubious ethical status, the gene encoding vancomycin resistance was transferred in the laboratory from a vancomycin-resistant enterococcus into a meticillin-resistant *Staphylococcus aureus*. In 1997, the first naturally occurring vancomycin-resistant, meticillin-resistant *Staphylococcus aureus* appeared in Japan. New antibiotics used in the treatment of MRSA include synergid - a combination of quinupristin and dalfopristin - and the new broad-spectrum tetracycline tigecycline. It remains to be seen how long will these remain effective.

Some antimicrobial resistance genes have been found located only on the bacterial chromosome. Others have been found on plasmids. Plasmids encoding antibiotic resistance are often called resistance factors, R-factors or R-plasmids. R-plasmids may encode resistance to several unrelated antibiotics. Some R-plasmids are self-transmissible and can move from strain to strain, even between different bacterial genera. Other R-plasmids, although not self-transmissible may be mobilized by other plasmids and that need not necessarily encode antibiotic resistance. Furthermore, antibiotic resistance genes are frequently located within transposons. These can move more or less at random around the bacterial genome. Genes encoded by transposons thus spread very easily because many transposable elements may become associated with transmissible plasmids. In this way, antibiotic resistance genes may become rapidly disseminated. Control of antimicrobial resistance in pathogenic microbes is one of the greatest challenges currently facing medical microbiology.

Summary

Antimicrobial agents can be divided into two categories based on their effects on target cells. Drugs that actually kill microorganisms are termed bactericidal. Drugs that only inhibit the growth of microorganisms are termed bacteriostatic. The decision to use a bactericidal or bacteriostatic drug to treat infection depends entirely on the type of infection. For example, bactericidal drugs will only kill cells that are actively growing. Bacteriostatic drugs, in comparison, will only inhibit the growth of cells. The bacteriostatic agents include streptomycin, aminoglycosides and penicillin where as sulphonamides, chloramphenicol and tetracyclines are bactericidal.

Antimicrobial agents can be classified into five categories by their range of activity. The first of these is termed *narrow spectrum*. Narrow spectrum drugs, as the name implies, are only active against a relatively small number of organisms. In general, narrow spectrum antibiotics are effective against Gram-positive organisms. The second comprising *moderate spectrum* drugs are

generally effective against the Gram-positives and most systemic, enteric and urinary tract Gram-negative pathogens. The beta-lactam antibiotics (penicillin, ampicillin, cephalosporins, etc.) belong to third category, *narrow and moderate spectrum* because some members are only effective against Gram-positive organisms while other members can also kill certain Gram-negative bacteria. A fourth category is termed *broad spectrum*. These drugs are effective against all prokaryotes with two exceptions: *Mycobacteria* (see below) and *Pseudomonas*. The fifth group includes those drugs that are effective against *Mycobacteria* (Table 2).

Table 2: Broader classification of antimicrobial agents on the basis of range of their action

S. No.	Spectrum/Range	Target organism(s)	Antibiotic
1	Narrow	Gram-positives (<i>Actinomyces</i> , <i>Corynebacteria</i> , <i>Bacillus</i> , <i>Clostridium</i> , pyogenic cocci, <i>Spirochetes</i>)	Macrolides (Erythromycin) Polypeptides (Polymyxin)
2	Moderate	Gram-positives plus systemic, enteric and urinary tract Gram- negatives	Sulphonamides and aminoglycosides (Streptomycin, Gentamycin, Tobramycin)
3	Narrow/moderate	Gram-positives plus Gram-negatives	Beta-lactams (Penicillin, Ampicillin, Cephalo-sporins)
4	Broad	All prokaryotes except <i>Mycobacteria</i> and <i>Pseudomonas</i>	Chloramphenicol, Tetracycline
5	Anti- mycobacterial	<i>Mycobacteria</i>	Ethambutol, Isoniazid, Streptomycin, Rifampin

Another method of classifying antimicrobial agents is by their site of activity within the target cell (Table 3). They may affect either the integrity or the synthesis of these sites. The various cellular targets include the cell wall, the plasma membrane, the nucleic acids and proteins.

Table 3: Antimicrobial agents and their site of action

Site of Activity	Example Antibiotics/ agents
Inhibition of cell wall integrity	Lysozyme
Inhibition of cell wall synthesis	
1. Biosynthetic enzymes (cytoplasmic)	Fosfomycin, Cycloserine
2. Membrane-bound phospholipid carrier	Bacitracin
3. Polymerization of subunits	Beta-lactams
4. Combine with wall substrates	Vancomycin
Inhibition of membrane integrity	Surfactants, Polyenes, Polypeptides
Inhibition of membrane synthesis	None
Inhibition of nucleic acid integrity	Alkylating, Intercalating agents (mitomycin, chloroquin)
Inhibition of nucleic acid synthesis	
1. Metabolism of DNA	5-Fluorocytosine, Acyclovir, NTP analogs
2. Replication of DNA	Nalidixic acid, Novobiocin, Nitroimidazoles
3. Synthesis of RNA	Rifampin
Protein integrity	Phenolics, Heavy metals
Protein synthesis	
1. 30S Subunit	Streptomycin, Kanamycin, Tetracycline
2. 50S Subunit	Chloramphenicol, Macrolides (Clindamycin, Erythromycin)
3. Folate metabolism	Sulfonamides, Trimethoprim

The problem of antibiotic resistance is becoming increasingly as more and more strains of pathogenic microorganisms are untreatable with commonly used antimicrobial agents. Researchers attribute this problem to a variety of factors including overuse of antibiotics in agriculture, animal husbandry and medicine and misuse of antibiotics. In addition, antibiotic resistance is often plasmid-borne, which means that resistance can readily be transferred from one organism to another. There are several mechanisms (Table 4) for antibiotic resistance and these relate to the sites of antimicrobial activity. These mechanisms include:

1. Altered receptors for the drug
2. Decreased entry into the cell
3. Destruction or inactivation of the drug

Table 4: Broader mechanism(s) of resistance to various antimicrobial agents

Altered Receptors	
1. Beta-lactams	Altered Penicillin Binding Proteins
2. Macrolides	Methylation of 2 adenine residues in 23S rRNA of the 50S subunit
3. Rifampin	Single amino acid change in RNA polymerase β -subunit
4. Sulfonamide/trimethoprim	Altered synthetase binds pABA preferentially/altered reductase for TMP
5. Nalidixic acid	Altered gyrase
6. Streptomycin	Altered S12 protein in 30S subunit
Decreased Entry	
1. Tetracycline	Normally biphasic, active transport reduced
2. Fosfomycin (chromosomal)	Glucose-6-phosphate transport reduced
Destruction/Inactivation	
1. Chloramphenicol acetyltransferase	Acetylates chloramphenicol
2. Beta-lactamases	Cleaves β -lactam ring
3. Aminoglycosides	Acetylation or phosphorylation as drug passes membrane

While antimicrobial agents can be life saving, they also sometime pose certain dangers to the patient. Some antibiotics are relatively safe; others should only be used if there is no other means of controlling an infection. The following table (Table 5) lists some side effects/ toxicity of antimicrobial chemotherapy.

Table 5: Toxic effects of antimicrobial agents

Side effects/ toxic effects	Examples
Overgrowth of pathogens	Intestinal (<i>C. difficile</i>), vaginal (<i>Candida</i>) pathogens.
Depression of intestinal symbiotes	Several
Nephrotoxicity	Polypeptides, Aminoglycosides
Ototoxicity - 8th cranial nerve	Aminoglycosides
Ophthalmic toxicity	Ethambutol
Aplastic anemia	Chloramphenicol
Hypersensitivity	Penicillin
Bone- seeking agents*	Tetracycline

*Bone- seeking agents are drugs characterized by high affinity for bone and are disposed in bone for prolonged periods of time while maintaining remarkably low systemic concentrations.

Common antiviral drugs

Drug	Uses	Mechanism of Action
Acyclovir, famciclovir, valaciclovir	Herpesviruses	Nucleoside analogue
Ganciclovir	Cytomegalovirus	Nucleoside analogue
Azidothymidine	HIV	Nucleoside analogue
Ribavirin	RS virus	Nucleoside analogue
Amantadine, rimantadine	Influenza A	Inhibit virus uncoating and assembly
Nevirapine	HIV	Protease inhibitor

Viruses use host cell machinery and are therefore very difficult to target.

Common antifungal agents

Drug	Uses	Mechanism of Action
<u>Amphotericin B</u>	Systemic fungal infections	Alters permeabilization of cell membrane
Azoles e.g. Clotrimazole, miconazole, fluconazole	Local candidosis and dermatophyte infections. Systemic fungal infections	Inhibit sterol synthesis
Flucytosine	Serious fungal infections	Competes with uracil

Antifungal agents tend to have a low therapeutic index and are rapidly destroyed by cellular enzymes.

Common antiprotozoal agents

Drug	Uses	Mechanism of Action
Imidazoles, e.g. <u>metronidazole</u>, tinidazole	Entamoeba, Giardia, Trichomonas	Interferes with several metabolic pathways
Pyrimethamine	Malaria, toxoplasma	Inhibits folic acid reduction
Pentamidine	Pneumocystis, Trypanosoma rhodesiense/gambiense	Inhibits aerobic glycolysis
Chloroquine, quinine	Malaria	Inhibits nucleic acid synthesis

Suggested Reading

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