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Bacteriostatic Agents

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1. Introduction

In this chapter we begin to study the effect of the antibacterial agents used for control of microbial growth

There are some essential related terms for studying the antibacterial agents that are mentioned as in following:

a. **Biocide**: A widespread chemical or physical agent which inactivates microorganisms.
b. **Bacteriostatic**: property of a specific biocide agent which is able to bacterial multiplication.
c. **Bactericida**l: A specific term referring to the property by which a biocide is able to kill bacteria.
d. **Disinfectants**: Products or biocides used to reduce only the number of viable microorganisms on the inanimate objects
e. **Septic**: Characterized by the presence of pathogenic microbes in living tissue.
f. **Antisepti**c: A biocide or product that inhibits the growth of microorganisms in or on living tissue.
g. **Aseptic**: Free of or using methods to keep free of, microorganisms.
h. **Antibiotics**: Naturally occurring or synthetic organic compounds which inhibit or destroy selective bacteria, generally at low concentrations.
i. **Sterilization**: is defined as the process where all the living microorganisms, including bacterial spores are killed. Sterilization can be achieved by physical, chemical and physiochemical means.
j. **Asepsis** is the employment of techniques (such as usage of gloves, air filters, uv rays etc) to achieve microbe-free environment.

Large numbers of antibacterial agents are of clinical interest. The mechanisms by which compounds with antibacterial activity inhibit growth or cause bacterial death are varied and depend on the affected targets. Some strategies of antibacterial agents are introduced as following:

Damage to DNA

Ultraviolet light, ionizing radiations and DNA-reactive chemicals are example of physical and chemical agents that act by damaging DNA. Among the DNA-reactive chemicals,

alkylating agents react covalently with purine and pyrimidine bases to form DNA interstrand cross-links. Ultraviolet light, for example, induces cross-linking between adjacent pyrimidines on one or the other of the two polynucleotide strands, forming pyrimidine dimers; ionizing radiations produce breaks in single and double strands.

Protein denaturation

The tertiary structure of the protein is readily disrupted by a number of physical or chemical agents, causing the protein to become nonfunctional. The disruption of the tertiary structure of a protein is called protein denaturation. A range of antibacterial agents inhibit the translation of the messenger RNA (mRNA) chain into its corresponding peptide chain.

Disruption of cell membrane

The cell membrane is known as a selective barrier, allowing some solutes to pass through and excluding others. Some bactericidal agents may alter the physical and chemical properties of the membrane, preventing its normal functions and therefore killing or inhibiting the cell. The structure of the cytoplasmic membranes in bacterial cells can be readily disrupted by certain agents. Polymyxins are the most important antibiotics which act on the membranes of Gram-negative bacteria. Amphotericin B and Nystatin are other toxic molecules named as polyene antifungal agents which have inhibitory action on membrane function

Disruption of cell wall

Destroying or preventing the synthesis of cell wall occurred after exposure to agents such as Lysozyme and Penecillin, respectively. The disruption of the cell wall may cause the cell lysis.

Synthesis of peptidoglycan precursors starts in the cytoplasm; wall subunits are then transported across the cytoplasmic membrane and finally inserted into the growing peptidoglycan molecule. Several different stages are therefore potential targets for inhibition. β-lactams , Bacitracin and Cycloserines are inhibitors of synthesis of peptidoglycan . β-lactams are the most important and the glycopeptides which are active only against Gram-positive bacteria. Cycloserines mainly used as a 'second-line' medication for treatment of tuberculosis, discussed later in this chapter have many fewer clinical applications.

Removal of free sulfhydryl groups

A large number of the antibiotics have demonstrated chemical reactivity toward compounds containing sulfhydryl groups. There have also been observed marked differences in reactivity of individual antibiotics toward various types of sulfhydryl-containing compounds.

Enzyme proteins containing cysteine have side chains terminating in sulfhydryl groups. In addition to these, coenzymes such as coenzyme A and dihydrolipoate contain free sulfhydryl groups. Such enzymes and coenzymes cannot function unless the sulfhydryl groups remain free and reduced. Oxidizing agents thus interfere with metabolism by forming disulfide linkages between neighboring sulfhydryl groups.

The most widespread methods used for controlling microorganism are the application of chemical and physical agents.

The most widely used physical methods include heat, radiation, and filtration which can destroy or remove undesirable microorganisms. Here we discuss how these methods work and discuss some practical examples.

Heat

One of the simplest means of sterilization is heat. Heat acts by oxidative effects as well as denaturation and coagulation of proteins. Those articles that cannot withstand high temperatures can still be sterilized at lower temperature by prolonging the duration of exposure. Dry heat acts by protein denaturation, oxidative damage and toxic effects of elevated levels of electrolytes. The moist heat acts by coagulation and denaturation of proteins. Moist heat is superior to dry heat in action. Temperature required to kill microbe by dry heat is more than the moist heat. The minimum time required to kill a suspension of organisms at a predetermined temperature in a specified environment is known as Thermal death time.

A temperature of 100°C will kill all bacteria, but in laboratory-scale cultures, within 2–3 minutes; a temperature of 121°C for 15 minutes whit 15 pound per inch is utilized to kill spores.

Radiation

Two types of radiation are used, ionizing and non-ionizing. Non-ionizing rays are low energy rays with poor penetrative power while ionizing rays are high-energy rays with good penetrative power.

Non-ionizing rays: Rays of wavelength longer than the visible light are non-ionizing. Microbicidal wavelength of UV rays lie in the range of 200-280 nm, with 260 nm being the most effective. UV rays are generated using a high-pressure mercury vapor lamp. It is at this wavelength that the absorption by the microorganisms is at its maximum, which results in the germicidal effect. UV rays induce formation of thymine-thymine dimers, which ultimately inhibit DNA replication. UV readily induces mutations in cells irradiated with a non-lethal dose. Microorganisms such as bacteria, viruses, yeast that are exposed to the effective UV radiation are inactivated within seconds. Since UV rays don't kill spores, they are considered to be of use in surface disinfection. UV rays are employed to disinfect hospital wards, operation theatres, virus laboratories, corridors, etc.

Ionizing rays: Ionizing rays are of two types, particulate and electromagnetic rays.

Electron beams are particulate in nature while gamma rays are electromagnetic in nature. High speed electrons are produced by a linear accelerator from a heated cathode. Electron beams are employed to sterilize articles like syringes, gloves, dressing packs, foods and pharmaceuticals.

Sterilization is accomplished in few seconds. Unlike electromagnetic rays, the instruments can be switched off.

Filtration

In the filtration method microbes do not kill, it just separates them out. Membrane filters with pore sizes between 0.2-0.45 µm are commonly used to remove particles from solutions that can't be autoclaved.

Various applications of filtration include removing bacteria from ingredients of culture media, preparing suspensions of viruses and phages free of bacteria, measuring sizes of viruses, separating toxins from culture filtrates, counting bacteria, clarifying fluids and purifying hydrated fluid. Different types of filters are Earthenware filters, Asbestos filters, Sintered glass filters, Membrane filters and Air Filters.

The other antimicrobial agents are those chemicals which destroy pathogenic bacteria from inanimate surfaces. They are listed in the table (1).

Chemical	Mode of action	Uses
Alcohols	Denaturing proteins and Solublizing lipids	Antiseptic used on skin
Formaldehyde (8%)	Reacting with NH_2, SH and COOH groups	Disinfectant, kills endospores
Tincture of Iodine	Inactivating the proteins	Antiseptic used on skin Disinfection of drinking water
Chlorine (Cl_2) gas	Formation of hypochlorous acid (HClO), a strong oxidizing agent	Disinfect drinking water; general disinfectant
Heavy metals	Inactivating the proteins	Disinfection of skin and laboratories
Mercuric chloride	Inactivation of proteins by reacting with sulfide groups	Disinfectant, although occasionally used as an antiseptic on skin
Detergents	Disruption of cell membranes	Skin antiseptics and disinfectants
Ethylene oxide gas	Alkylating agent	Disinfectant used to sterilize heat-sensitive objects such as rubber and plastics
Ozone	Produces lethal oxygen radicals	Purification of water, sewage
Phenols	decreasing the surface tension	Disinfection of laboratory devices, toilet and recycle bin

Table 1. Chemical antibacterial agents

Antibiotics fight against bacteria by inhibiting certain vital processes of bacterial cells or metabolism. Based on these processes, we can divide antibiotics into five major classes:

1. Cell wall inhibitors, such as Penicillin and Vancomycin.
2. Inhibitors of cell membrane function, such as Polymyxin B and Daptomycin.
3. Protein synthesis inhibitors, such as Aminoglycoside.

4. Inhibitors of nucleic acid synthesis, such as Fluoroquinolones, which inhibits DNA synthesis, and Rifampin, which inhibits RNA synthesis.

Inhibition of cell wall synthesis

The cell wall contains chemically distinct polysaccharides. The polysaccharides contain the amino sugars *N*-acetyl glucosamine (GlcNAc) and acetylmuramic acid (MurNAc). All β-lactam drugs are selective inhibitors of bacterial cell wall synthesis and therefore active against growing bacteria.

The bacterial cell wall-a unique structure in most bacteria can be affected in several ways: at different stages of synthesis (Fosfomycin, Cycloserine) or transport (Bacitracin, Mureidomycins) of its metabolic precursors, or by a direct action on its structural organization (β-lactams, Glycopeptides). The initial step in drug action is binding of the drug to cell receptors (Penicillin-binding proteins; PBPs). β-lactam drugs act as a false substrate for D-alanyl-D-alanyl transpeptidases , so they inhibit the transpeptidation reaction and peptidoglycan synthesis. In the next step, inhibitor of autolytic enzymes in the cell wall is inactivated. This activates the lytic enzyme and results in lysis if the environment is isotonic. So, β-lactam drugs are only active against rapidly dividing bacteria and growth lag phase ones are more stable to cell wall synthesis inhibitors.

Penicillins, Cephalosporins, Vancomycin, and Cycloserine inhibit the cell wall synthesis. Several other drugs, including Bacitracin, Teicoplanin, Vancomycin, Ristocetin, and Novobiocin, inhibit early steps in the biosynthesis of the peptidoglycan. In an effective inhibitory mechanism these drugs must be penetrated in the early stages of the cell wall synthesis took place inside the cytoplasmic membrane. In the case of Glycopeptides such as Vancomycin and Teicoplanin, attachments to D-ALA-D-ALA terminal end of peptidoglycan precursors occur. This inhibits the action of transglycosidase and transpeptidases, resulting in cell wall impairment.

The difference in susceptibility of gram-positive and gram-negative bacteria to various Penicillins or Cephalosporins would be attributed to the structural differences in their cell walls. Transpeptidases are located in periplasmic space that is directly accessible in gram-positive bacteria but not in Gram-negatives; so, theses drugs need to cross the outer bacterial cell membrane of Gram-negatives (passive diffusion) or pass through porin channels.

Some factors (eg, amount of peptidoglycan, presence of receptors and lipids, nature of cross-linking, activity of autolytic enzymes) affect the penetration, binding and activity of the drugs

Inhibition of cell membrane function

The cytoplasmic membrane is a selective permeability barrier, carries out active transport functions, and thus controls the internal composition of the cell. Macromolecules and ions can escape from the membrane as a result of cytoplasmic membrane disruption or cell damage. The cytoplasmic membrane of bacteria and fungi is more rigid than animal or plant cells and can be disrupted by certain agents. Consequently, selective chemotherapy is suggested.

Amphotericin B, Colistin, Ionospheres, Daptomycin , the Imidazoles and Triazoles are other examples of agents which inhibit the function of cell membrane. The detail mechanisms of action of other cell membrane inhibitors are shown in the table (2).

Inhibition of protein synthesis

Protein synthesis can be blocked by a large variety of compounds that affect any of the phases of this process, including activation (Mupirocin), initiation (Oxazolidinones, Aminoglycosides), binding of the tRNA amino acid complex to ribosomes (Tetracyclines, Glycylcyclines) and elongation (Amphenicols, Lincosamides, Macrolides, Ketolides, Streptogramins, Fusidic acid). In details, Tetracycline, Minocycline and Doxycycline, reversibly bind to the 30S subunit of ribosome and inhibit binding of aminoacyl-t-RNA to the acceptor site (A-site) on the 70S ribosome. Aminoglycosides also, bind to the A-site of the 30S subunit (the equivalent of mammalian 40S subunit) in an energy dependent process. In contrast to Tetracycline, the binding of Aminoglycosides to the A-site of the 30S subunit is irreversible. This mode of action means that Aminoglycosides act as bactericidal agents while Tetracycline belong to bacteriostatic agent group. This frustrating binding, freeze the 30S initiation complex (30S-mRNA-tRNA), disturbs elongation of the peptide chain. At the second step, aminoglycosides impair translational accuracy that finally lead to misreading of the mRNA sequence and/or premature termination of protein synthesis.

On the other hand, the large subunit of bacterial ribosomes (the equivalent of mammalian 60S subunit) occupied by Macrolides and some non-macrolides such as Chloramphenicol and Lincosamides. Premature dissociation of peptidyl tRNA from ribosome during elongation process occurred base on the attachment to 23S rRNA of the 50S ribosomal subunit. Consequently, peptidyl tRNA translocation from A to P site inhibited and peptide bond formation would be blocked; so, truncated peptide would be released after that. Similar mechanism is used by Lincosamides (Lincomycin and Clindamycin) that bind 50S subunit of ribosomes to inhibit protein synthesis.

Protein synthesis is also inhibited by another Macrolide (erythromycin) with a completely different way. Erythromycin prevents assembly of 50S subunit and as a result, no functional ribosome emerged that could trigger protein synthesis. This mechanism is also used by a new class of synthetic antibacterials (Linezolid) that inhibit the formation of the initiation complex.

Fusidic acid binds to elongation factor G (EF-G) and inhibits release of EF-G from the EF-G/GDP complex.

Rifampin, Rifamycin, Rifampicin bind to DNA-dependent RNA polymerase and inhibit initiation of RNA synthesis.

Inhibition of nucleic acid synthesis

Examples of drugs acting by inhibition of nucleic acid synthesis are the Quinolones, Pyrimethamine, Rifampin, Sulfonamides, Trimethoprim, and Trimetrexate.

Nitroimidazoles, Nitrofurans affect DNA directly. Trimethoprim and Sulfamides block bacterial metabolic pathways. Some compounds are unable to kill bacteria but can block

bacterial mechanisms of resistance, enhancing the activity of other antimicrobials administered in combination. Among this group of agents, only certain β-lactamase inhibitors are currently in clinical use.

All Quinolones and Fluoroquinolones inhibit microbial DNA synthesis by blocking DNA gyrase.

In the following table are grouped characteristics of each class of antibiotics and mode of action.

Class	Example	Mode of action
Aminoglycoside	Gentamicin,Tobramycin,Amikacin	Bactericidal; inhibit protein synthesis
β-lactam/β-lactamase inhibitors	Ampcillin-sulbacam, Ticaracillin-clvulnate, Piperaciin-Tazobactam	Bactericidal; inhibit cell wall synthesis
Cephalosporin	Cefotaxime,Ceftriaxone, Ceftazidime, Cefepime	Bactericidal; inhibit cell wall synthesis
Fluoroquinolone	Levofloxacin, Ciprofloxacin, Moxifloxacin	Bactericidal; block DNA replication
Glycopeptide	Vancomycin	Bactericidal; inhibition of cell wall synthesis
Glycylcycline	Tigecycline	Bacteriostatic; inhibit protein synthesis
Macrolide	Erythromycin,Clarithromucin, Azithromycin	Bacteriostatic; inhibit protein synthesis
Oxazolidinone	Linezolid	Bacteriostatic; inhibit protein synthesis
Polymyxins	Polymyxin B, Colistin	Bactericidal; disrupt cell membrane
Tetracycline	Doxycycline, Tetracycline, Minocycline	Bacteriostatic; inhibit protein synthesis

Table 2. Antimicrobial agent classification and mode of action

Resistance to antimicrobial drugs

Infectious microorganisms can develop ways to exhibit resistance to drugs. This antibiotic resistance is due to the increasing use of antibiotics. There are many different mechanisms by which microorganisms can survive. Acquired resistance is often caused by mutations in chromosomal genes, or by the acquisition of mobile genetic elements, such as plasmids or transposons, which carry the antibiotic resistance genes.

1. Production of destroying enzyme

Organism may acquire genes encoding enzymes, such as β-lactamases, that destroy the antibacterial agent before it can have an effect. This enzyme destroys and inactivates the penicillin G drug. An important strategy of organisms for resistance to penicillins is due to

penicillin-destroying enzymes (β -lactamases).β -Lactamases disrupts the antimicrobial activity of penicillins and cephalosporins by opening the β -lactam ring. Some inhibitors that have a high affinity for β -lactamase are Clavulanic acid, sulbactam, and tazobactam. Gram-negative bacteria produce some adenylylating, phosphorylating, or acetylating enzymes for resistant to aminoglycosides

2. Altering the permeability of the drugs

Bacteria may acquire efflux pumps that extrude the antibacterial agent from the cell before it can reach its target site and exert its effect. For example, changing the permeability of the drug (e.g Polymyxins) is one of the strategies of organism to exhibit resistance.

3. Altering the structural target for the drug

Bacteria may acquire several genes for a metabolic pathway which ultimately produces altered bacterial cell walls that no longer contain the binding site of the antimicrobial agent. Organisms which are resistant to erythromycin alter the receptor on the 50S subunit of the ribosome through methylation of a 23S ribosomal RNA. The loss or alteration of PBPs is another resistance mechanism of some drugs (e.g. Penicillins and Cephalosporins).

4. Altering the metabolic pathway

Some Sulfonamide-resistant bacteria do not require extracellular PABA but can utilize preformed folic acid.

5. Altering the function of enzyme

that can still perform its metabolic function but is much less affected by the drug. In Trimethoprim-resistant bacteria, the dihydrofolic acid reductase is inhibited far less efficiently than in trimethoprim-susceptible bacteria.

Factors affecting antimicrobial activity

Some antimicrobial agents are microbicidal under one set of conditions and microbistatic under others. Factors that influence the activity of antimicrobial agents are (1) the susceptibility of the microorganism, (2) the concentration or dose of the agent, (3) the length of exposure, (4) the number of microorganisms, and (5) environmental conditions.

Microbial susceptibility

Microbes vary in their response to different disinfectants. For example, vegetative cells of the Mycobacteria that cause tuberculosis and leprosy, however, are covered by a waxy coating that protects them from many antimicrobial chemicals. In addition, the hepatitis B virus and some fungal spores are resistant to most disinfectants and are persistent problems in hospitals. *Bacillus* and *Clostridium* are especially difficult to eliminate.

Concentration or dose of the agent

Diluting microbicidal chemicals usually weakens their antimicrobial activity. At lower concentrations they become microbistatic or lose antimicrobial activity altogether. The antimicrobial effects of temperature or radiation also depend on intensity of the exposure.

Low dose may inhibit growth, whereas high doses may result in sterilization. With a few important exceptions, the more concentrated, the more target organisms will be destroyed.

Length of exposure

Microorganisms die when physical or chemical conditions irreversibly damage essential cell components. All organisms present, however, do not die rapidly and simultaneously when a critical exposure is achieved, because microbial death is a function of time-the longer microbes are exposed to potentially lethal conditions, the more microbes will be killed. For many germicides, if exposure time is long enough, the probability of even a single cell surviving becomes so low that sterilization is practically assured. In contrast, microbistatic agents are effective only as long as they are present and must be used during the entire time that inhibition is to be maintained.

Number of microorganisms

Antimicrobial effectiveness also depends on the initial concentration of the microbial population. As the number of microbial contaminants increases, either the exposure period to or concentration of the agent must increase to achieve acceptable levels of decontamination.

Environmental conditions

Temperature, pH, and moisture affect the efficiency of most antimicrobial agents. In addition, some chemical agents are absorbed by organic materials (blood, mucus, feces, and tissue) that severely reduce antimicrobial effectiveness. These agents therefore cannot be used on the skin. Some antimicrobial agents are impeded by soaps and detergents that remain as thin films on skin or object surfaces. This difficulty can be avoided by through rinsing prior to disinfection or antisepsis.

Enzybiotics

The heavy use of antibiotics during the last century has resulted in widespread bacterial resistance. Overcoming resistance requires the development of antibiotics aimed at new targets in microorganisms. Preferably, such targets should be highly conserved in bacteria and required for pathogenesis, but not found in humans.

One of the most recently delivered classes of antibiotics are enzybiotics, the lytic enzymes named because of their enzymatic mode of action in degrading bacteria. Originally, enzybiotics named bacteriophage lytic enzymes that destroy the cell wall of the host bacterium quickly. According to the nature of bacteriophages that select their host specifically, the bacteriophage lytic enzymes were so specific. As the first attempt, Nelson and co-workers designate an enzybiotics for fighting with group A *Sreptococcus pyogenes* (*S. pyogenes*), the primary etiologic agent of bacterial pharyngitis (an inflammation of the throat or pharynx). But we must mention that, enzybiotics refer to all kind of enzymes with any kind of sources that have the ability to overcome bacterial infection.

The nick name of enzybiotics is peptidoglycan hydrolyses which induced the enzymatic cleavage of peptidoglycan covalent bonds of bacterial cells. As we know, the backbone of

peptidoglycan consists of alternating residues of GlcNAc and MurNAc. The tetrapeptide side chains branching off from MurNAc are cross-linked by the pentaglycine bridges. This is the major mode of enzybiotics action that leads to the hypotonic lysis of poor bacteria by degrading different parts of their protective cell wall. Here, we would discuss the well-known enzybiotics according to, their site of invasion to the peptidoglycan backbone of bacteria.

1. Amidase enzybiotics

Lysins are the major class of Amidase enzybiotics that work on covalent bonds of bacterial peptidoglycan. They are the products of bacteriophages double-stranded DNA and named endolysin as a result of their non-bacterial sources. Another class of Amidase enzybiotics is autolysins that emerge from host bacterium. Both classes could fall in to three sub-classes according to their location of bond cleavage in bacterial peptidoglycan backbone. i) *N* - acetylmuramoyl- L- alanine amidases that break up the covalent bond between MurNAc and the first amino acid (L- alanine) of tetrapeptide side chain. ii) endopeptidase that break up the covalent bond between internal amino acids of tetrapeptide side chain especially between the first L- alaninen and the second D- Glu. Another endopeptidase act on pentaglycine bridges and dissociate the internal links of the backbone. iii) the third brother enjoy cleavage of covalent bond between *N* - acetylglucosamine and *N* - acetylmuramic acid by muramidases, transglycosylases and glucosaminidases activities (Figure1).

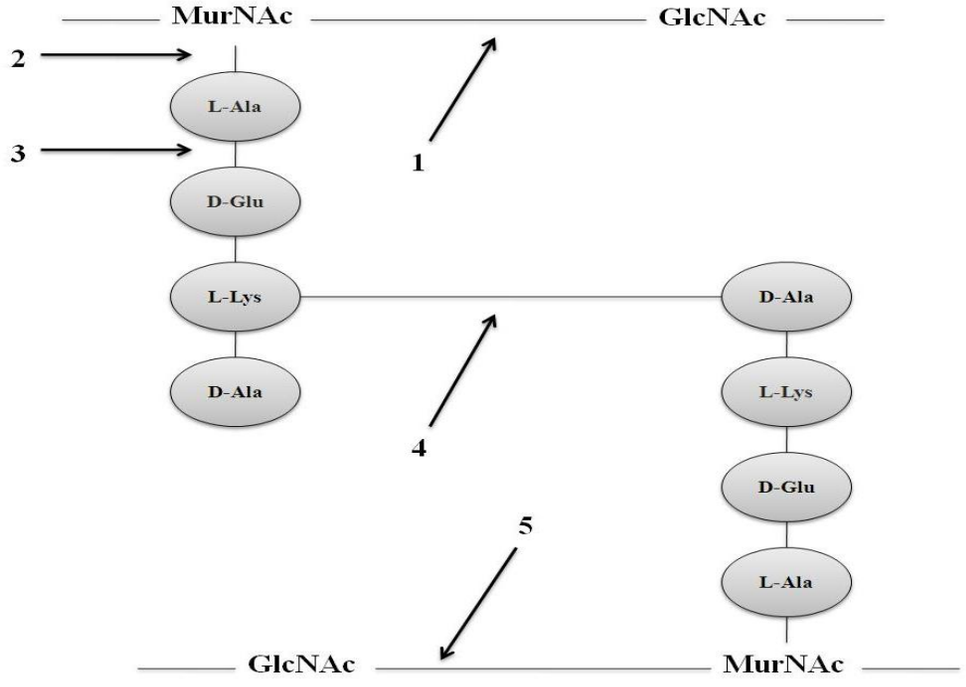

Fig. 1. The sites of cleavage by main classes of enzybiotics are shown by the numbered arrows (1) muramidases and transglycosylases; (2) amidases; (3 and 4) endopeptidases; (5) glucosaminidases

One surprise point in applying lysins as new antibiotic is its strain specific function. For example Pal only lyses pneumococcal strains while Ply3626 of kills *Clostridium perfringens*

(*C. perfringens*) strains. The most potent lysine discovered in group C streptococci C1 phage named PlyC Amidase first reported in 1957. PlyC Amidase is the only known multimeric lysine that did not act on streptococci groups B, D, F, G, L, and N so, it is limited to pathogenic streptococci groups A and C. Pal1, encoded by pneumococcal bacteriophage Dp-1, lyses Penicillin-resistant *Streptococcus pneumoniae* strains as well as penicillin- sensitive ones. Cpl-1 muramidase ia another anti-pneumococcal enzyme that came from Cp–1 phage. A broad range lysin (PlyGBS lysin) was also reported in *Streptococcus agalactiae* bacteriophage NCTC 1126. PlyGBS lysin contain two endopeptidase and muramidase domains that enable it efficient against groups A, C, G, and L streptococci. The other broad range lysin that kills groups A, B, C, E, G streptococci and also *Enterococcus faecalis* is B30 lysin. B30 lysin also includes two previous domains and extracted from *S. agalactiae* B30 phage. Other antibacterial lysins are found in *Staphylococcus aureus*, coagulase-negative staphylococci, *Bacillus anthracis*, *Bacillus cereus*, *Listeria monocytogenes*, *C. perfringens* phages and one of them (PlyG lysine) is used for the identifi cation of *B. anthracis* by the U.S. Centers for Disease Control and Prevention.

2. Endopeptidase enzybiotics

Endopeptidase or endoproteinase are proteolytic peptidases that in contrast to exopeptidases, break peptide bonds of nonterminal amino acids. Lysostaphin and zoocin A are two endopeptidase encoded by *Staphylococcus simulans* biovar *staphylolyticus* and *Streptococcus equi* ssp.

3. *N*-acetylmuramidases enzybiotics

Lysozymes, or *N* - acetylmuramidases, are produced by cells of many different animal species, plants, insects, bacteria, and viruses. Lysozymes are unique antibiotics, so that they contain enzematic and also non-enzematic mechanisms for fighting with foreign bacterias. There are some facts about lysosymes take make them so different from other enzybiotics. First of all, Lysozymes are the only peptidoglycan hydrolases that have been used on a larger scale in humans for the past several decades. Second, antibacterial action of lysozyme is based on the enzymatic cleavage of peptidoglycan, and nonenzymatic mechanisms based on activation of autolysins and also cytoplasmic membrane destabilization resulting from the removal of divalent ions from the membrane surface.

Plants antibiotics

Traditionally, people get used to herbal treatment as a natural way to fight diseases almost in all continents. Some of them take it easier to go to herbalist than a doctor, and also prefer natural drugs than chemically synthesized ones. Especially, in ancient civilizations such as Iran, Iraq, India and China it has been so common to use plants as an effective tool for diseases therapy. Hippocrates (in the late fifth century B.C.) mentioned 300 to 400 medicinal plants. Avicenna (Ibn Sīnā) in his famous book "*The Canon of Medicine*" lists 800 tested drugs, including plant and mineral substances, and describe their specific properties according to the known diseases of that time.

Plants antibiotics come in to view after the arrival of antibiotics in the 1950s, when scientists realize that new antibiotics might be obligatory because of the antibiotic resistance. Major classes of plants antimicrobial components are phenolics, terpenoids, essential oils,

alkaloids, lectins and polypeptide and polyacetylenes. For example, the roots of *Glycyrrhiza glabra* contain glabridin and hispaglabridin B. The former is active element against both *Mycobacterium tuberculosis* H37Ra and H37Rv strains at 29.16 g/mL concentration. It was also clear that, glabridin was more active against Gram-positive strains than Gram-negative. Antimycobacterial activity of glabridin like component (hispaglabridin B) did not find because of their structural differences. Glabridin have two free phenolic hydroxyls which might be crucial in antibacterial activity while hispaglabridin might be inactivated after the corporation of one hydroxyl group in protected benzopyrene ring. Methicillin resistant *Staphylococcus aureus* (MRSA), and, *Helicobacter pylori* (*H. pylori*) are also sensitive to Glabridin. Anti-*H. pylori* activities are also shown in *rachyspermum copticum* and *Xanthium brasilicum* with minimum inhibitory concentrations within the range of 31.25-250 micro g/ml.

Grabidin strongly inhibits adenosine 3', 5'-cyclic monophosphate (cAMP) phosphodiesterase. Glabridin is associated with reduction in protein kinase C (PKC) activity and since PKC is required for low density lipoprotein (LDL) oxidation; so, grabidin induces reduction of LDL oxidation. This phenomenon is considered to be of major importance in atherosclerosis attenuation because LDL is associated in early atherogenesis. Glabridin can inhibit both mono- and diphenolase tyrosinase activities and because of the involvement of tyrosinase in melanin biosynthesis, glabridin may serve as candidates for skin-lightening agents. On the other hands, grabidin could serve as whitening agents for treatment of various dermatological disorders (melasama, age spots, and sites of actinic damage) that arise from the excessive accumulation of epidermal pigments. Cytochrome P450 3A4 enzymes is the major human drug metabolizing enzyme, that inactivated by glabridin antioxidant irreversible. The effect on P450 enzymes inactivation may play a role in the reported antiatherosclerotic activity of glabridin.

One of the most exciting plants with antibiotic activity is genus *Allium with* common garlic (*Allium sativum* L.) that known as Russian penicillin. In a study, two Allioideae alkaloids, canthin-6-one and 8-hydroxy-canthin-6-one, are extracted from *Allium neapolitanum*. They displayed minimum inhibitory concentrations (MICs) in the range 8-32 microg/mL against a panel of fast-growing Mycobacterium species and 8-64 microg/mL against multidrug-resistant (MDR) and MRSA. Antibacterial activities of *Eucomis autumnalis* and *Cyathula uncinulata* against ampicillin-resistant and kanamycin-resistant strains of *E.coli revealed a low MIC range of 0.27 mg/ml and 0.39 mg/ml respectively.*

New candidate antibiotics for MRSA are from Leguminosae family and the most active ones are the flowers of *A. auriculiformis* and *B. kockiana*. Some of these medicinal plants, such as *B. kockiana*, *B. purpurea*, *C. pulcherrima*, and *C. surattensis* have been used traditionally to treat various diseases in Malaysia. In the case of *A. auriculiformis* two acylated bisglycoside saponins, acaciaside A and B isolated, were found to exhibit antibacterial and antifungal activity.

In a study of resistant and standard strains of *Escherichia coli (E. coli)*, it has been reported that *Anagyris foetida* (Leguminosae) and *Lepidium sativum* (Umbelliferae) enhanced the activity of amoxicillin against resistant *E. coli* strain. Clarithromycin in combinations with *Gundelia tournefortii* L. (Compositae), *Eruca sativa* Mill. (Cruciferae), and *Origanum syriacum* L. (Labiateae), shows enhanced activity against the resistant *E. coli* strain. This strategy, the use of herbals and drugs in a multi targeted approach, named "herbal shotgun" or

"Synergistic multi-target effects". As a result, herbal-drug combinations affect not one but several targets, cooperating in an agonistic-synergistic way.

Umbelliferae family is known as an herbal antibiotic family after its comparison with standard antibiotics. The results show that cefixime and chloramphenicol resistant *Enterococcus faecalis* and *Pseudomonas aeruginosa* are sensitive to *Anethum graveolens*, *Foeniculum vulgare* and *Trachyspermum ammi*.

In a study of folk medicine, the effects of traditional therapeutic plants used by Haudenosaunee peoples of New York State have been administered. Four predicted plants (*Achillea millefolium, Ipomoea pandurata, Hieracium pilosella*, and *Solidago canadensis*), showed antimicrobial properties strongly against *Salmonella typhimurium*.

Strong attempts have been performed for treatment of brucellosis, a high morbidity zoonosis caused by brucella, with natural antibiotics in Iran. Among four effective herbs tasted as antibacterial agents against *Brucella melitensis (B. melitensis)*, *Oliveria decumbens* was chosen as the most effective plant for further studies. A *B. melitensis* strain that show resistance to tetracycline, nafcillin, oxacillin, methicillin, and colistin, inhibited by methanolic extract of *Oliveria decumbens* after 7 hours. Synergistic effect between *Oliveria decumbens* extracts and two other anticiotics (doxycycline and tetracycline) indicated as well.

Cuminum cyminum L. from Apiaceae family has been used since many years in Iranian traditional medicine. This aromatic herb is an astringent that has been used in the treatment of mild digestive and bronchopulmonary disorders as well as a cough remedy or analgesic. It has been shown that cumin seed essential oil significantly enhance antibacterial efficacy of ciprofloxacin against *Klebsiella pneumoniae* (*K. pneumoniae*). The authors theorize that essential oil damage cell wall or it modify the outer membrane proteins which lead to enhancement of ciprofloxacin activity against *K. pneumoniae.* As a result, they believe that herbal shotgun approach may be used in the case of cumin seed essential oil in future as a semi-natural way for fighting with *K. pneumoniae related disorders*.

Another medicinal plant that used extremely among Iranian population is *Zataria multiflora* (*Z. multiflora*) that belongs to the Lamiaceae family. Iranian traditional folk remedies, mainly used *Z. multiflora* as an antiseptic, analgesic, and carminative. In a wide local study of both gram-positive and gram-negative bacteria with important clinical impacts, some exciting results have been emerged. In the case of gram-positive cocci, and in presence of *Z. multiflora* essential oil (0.44 to 1.41 µL/mL) growth inhibition of both MRSA and MSSA has been observed. Same results with higher essential oil MICs have been reported for vancomycin-resistant E. faecalis (VREf) and vancomycin-sensitive E. faecalis (VSEf).

The growth of *E. coli* O157:H7, the cause of many food-borne outbreaks in different countries, inhibited at essential oil concentrations of 0.12 µL/mL for one ecotype of *Z. multiflora*. Two other gram-negative bacteria, *Salmonella entrica* and *Shigella flexneri*, inhibited and also killed at concentrations ranging from >0.12 to 2 µL/mL.

A phosphorylated structure, similar to the adenine, was isolated from the berries of *Solanum incanum (S. incanum)* Linnaeus *(*Thorn Apple or Bitter Apple*).* It was astonishing that crystals of this compound inhibit the growth of gram-positive and gram-negative bacteria, yeasts, dermatophytes, and some agricultural pathogens effectively. The zone of inhibition for 6.5mm diffusion disks was between 15-26mm with the highest inhibition for *S. pyogenes*

(26mm), *C. perfringens* (25mm) and *Clostridium septicum* (25mm) in bacterial group. It has been shown that, *S. incanum* crystals contain steroidal glycoalkaloids, solanine, which may act as a saprogenic surface active agent at high concentration. It must mensioned that solanine is found mainly in any part of solanum family plants (solanacea), including the leaves, fruit, and tubers and is rather high in the green peel and the sprouts.

Phage therapy, a candidate for antibiotic replacement

Phage therapy means the use of lytic bacteriophages as an alternative to antibiotics especially against the infection of resistant bacteria. Bacteriophages are bacterial viruses that invade bacterial cells and abberated as "phages". Phages have a developmental cycle within the host bacteria which can be lytic or lysogenic. The former involves a series of events that lead to the lysis of bacterial cell, but the lysogenic cycle comprises replication of phage nucleic acid together with the host genes for several generations.

There are some important benefits for phage therapy such as host-specific that did not observed in the case of routine antibiotics. Bacteriophages specificity could show important impacts in clinical use. Another advantages for phage therapy is its lower side effects and lesser therapeutic dose according to phages self-replicating in its target bacterial cell. Therefore, phage therapy is harmless to the eukaryotic host undergoing therapy theoretically.

Some bacteriophages synthesize degrading enzymes that breakdown the biofilms of bacteria that facilitate the bacterial cell lysis. Bacterial resistance to phages, if emerged, could be overcome according to the fact that mutation of phages occur with the same rate as bacteria. Cheap production of fighting bacteriophages is another advantage of

It must be mentioned that, phage therapy suffer from serious problems that make it unrealable.

Selection of appropriate mixture of high virulence phages against the target bacteria, poor understanding of heterogeneity and ecology of both the phages and the bacteria, are the most important problems. Resistance of bacteria to lysis by phages was another important challenge especially in the case of Pseudomonas plecoglossicida.

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