The Antimicrobial Age

**Introduction to Antimicrobials**

In 1885, German physician Paul Ehrlich published a work called “On the requirement of the organism for oxygen.” He described his idea of cells having side chains that could bind to oxygen molecules and support the cell’s function. Ehrlich hypothesized these side chains demonstrated specificity in the same way a key is specific to a lock - in other words the receptors on the side chains bound to only certain molecules while ignoring others.

At the same time, Ehrlich’s research focused on identifying dyes that could be used to distinguish one cell type from another. He used this technique to diagnose cases of pernicious anemia, a condition in which the number of red blood cells decreases because the intestines do not absorb enough vitamin B12. As Ehrlich continued to experiment with the dyes, he realized that the cells’ side chains might be tricked into mistaking the dyes, and even toxins, for the nutrients they normally bound. With his coworker Sahachiro Hata, Ehrlich began testing chemicals on mice infected with the microbe responsible for sleeping sickness. When the team tested a drug called “Preparation 606,” they found it effectively cured their mice. They eventually modified the drug to treat humans and in 1912 Neosalvarsan became commercially available to treat the sexually transmitted infection syphilis. Ehrlich and Hata’s discovery marked the beginning of a new era of discovery in medicine.

Many people use the terms “antibiotic” and “antimicrobial” interchangeably. Both indicate compounds used to combat bacterial infections, but the word “antibiotic” describes a substance produced naturally that has been harnessed for use in medicine, while antimicrobial is a broader term that can be accurately applied to any substance, whether made naturally or in a laboratory, for use in controlling infections. Neosalvarsan, which is a chemically modified arsenic-based compound, qualifies as an antimicrobial. Penicillin, arguably the best-known antimicrobial, can be called an antibiotic.

Before the advent of penicillin, even small cuts and scratches could be life-threatening if they induced blood poisoning. Even the introduction of Neosalvarsan could not limit the hazard of small wounds as it often lead to severe side effects. The story of the discovery of penicillin is well known and often serves to remind modern inventors that not all mistakes are detrimental. The scientist Alexander Fleming was in his laboratory sorting through petri dishes he had been growing bacteria on, when he found a plate contaminated with mold. Interestingly, the bacteria had not grown near the mold. Fleming hypothesized the “mold juice” was killing the bacteria and he and his team worked to isolate more mold and test its therapeutic benefits, but the compound was too unstable. Ten years later, Ernst Chain and Howard Florey resumed attempts to isolate the substance. Using methods devised by Chain and Florey and supported by funding from pharmaceutical firms worldwide, production of penicillin increased enough that it began to be used to treat patients. By 1943, the U. S. military began investing in penicillin production in order to treat soldiers wounded in World War II. In the same decade, tuberculosis (TB) alone accounted for nearly 4% of deaths in the United States. TB was also known as consumption because of the way people stricken with the disease coughed unceasingly and became severely underweight before they died. In 1943, Dr. Selman Waksman isolated bacteria from the soil that produced a compound he later name streptomycin. Streptomycin was the first effective treatment and cure for TB, and with this discovery, the antimicrobial era had arrived in full force.

**General Characteristics of Antimicrobials**

The usefulness of antimicrobials can be measured using the therapeutic index. The therapeutic index is the ratio of the highest dose of the drug that is not toxic to the patient to the amount of drug required to produce the desired effect. A high therapeutic index indicates a drug will be well-tolerated by most people, but a low therapeutic index means a patient being treated with the drug should be carefully monitored for adverse effects. Antimicrobials are designed to have as large a therapeutic index as possible, often by exploiting differences between human and bacterial cells. One classic antimicrobial target is the cell wall, which is present in many bacterial cells but is absent in human cells. This concept can also be described as selective toxicity meaning the drug kills or inhibits the microbes while damaging host cells as little as possible.

Antimicrobials can most broadly be described as either bactericidal or bacteriostatic. Bactericidal agents cause disruption and death of microbial cells. The drugs generally act on the bacterial cell wall, cell membrane, or DNA. Bacteriostatic substances only stop the bacterium from replicating without killing it and typically affect the microbe’s ability to synthesize proteins.

Finally, antimicrobials are often labeled as either narrow-spectrum or broad-spectrum. Broad-spectrum drugs are effective against a wide range of pathogens and can sometimes be used to treat infections that have not been definitely diagnosed. Narrow-spectrum drugs, in contrast, are effective only against a limited number of pathogens. Generally, naturally produced antibiotics affect a narrower range of bacteria than the semi-synthetic drugs derived from naturally produced compounds. Penicillin G, for example, is produced naturally but has a narrower range of efficacy compared to the penicillin derivative amoxicillin.

**Classes and Mechanisms of Antimicrobials**

*Cell Wall Synthesis Inhibitors*

A key difference between human and bacterial cells is the complete absence of a cell wall in human cells. Bacteria can be separated into two groups: Gram-positive and Gram-negative. Gram-positive bacteria have thick cell walls made of a substance called peptidoglycan. Gram-negative bacteria also have cells walls, but the peptidoglycan layer is much thinner compared to Gram-positive bacteria. The peptidoglycan forms a mesh-like structure on the cells surface. Cross-links form between adjacent peptidoglycan strands to form the strong cell wall (Fig. 1). The key enzyme involved in forming these cross-links is the penicillin-binding protein (PBP). Because cell walls are only found in bacteria, PBPs are also unique to bacteria. As a result, PBPs and other components of the cell wall are popular targets for antimicrobials.

 Penicillin and its derivatives, along with cephalosporins and carbapenems, belong to a class of cell wall synthesis inhibitors called beta-lactam drugs. The active portion of these drugs is a square called a beta-lactam ring, and they usually work by preventing bacteria from producing a cell wall. The beta-lactam ring is similar in structure to PBPs, which are the molecules that form the cross-links between peptidoglycan strands. When the cell attempts to construct a cell wall the beta-lactam rings in the antimicrobials compete for binding sites with the PBPs. When the PBPs bind the antimicrobial instead of the correct cross-link molecules, the cell wall loses its integrity and the bacterial cells die. Beta-lactam drugs are bactericidal but are only effective against bacteria that are actively growing and synthesizing new cell wall components. They are also considered broad-spectrum as they can be used effectively against both Gram-positive and Gram-negative bacteria.

 Vancomycin kills Gram-positive bacteria by affecting cell wall synthesis, but it is not a beta-lactam drug. Instead, vancomycin is a glycopeptide antibiotic meaning the molecule is a protein bonded to a sugar. The protein portion of the molecule binds to a sequence on the peptidoglycan and prevents the backbone of the molecules from forming. Vancomycin is often considered the drug of last resort in treating cases of life-threatening *Staphylococcus aureus* infections, but the rising occurrence of vancomycin-resistant *S. aureus* (VRSA) strains poses a major public health threat.

 Cell wall synthesis inhibiting antibiotics have high therapeutic indices, because they target a component of the bacterial cell that is missing in human cells. Because cell walls are found in bacteria but not people, treating infections with antimicrobials with this mechanism is an excellent way to target pathogens without damaging the host.

*Protein Synthesis Inhibitors*

Like cell wall synthesis inhibitors, protein synthesis inhibitors exploit a difference between eukaryotic (human) cells and bacterial cells. Ribosomes, the cellular machines that manufacture proteins, are made of different components in bacteria compared to human cells. Most protein synthesis inhibitors are bacteriostatic, because stopping proteins from being made usually does not kill the cell.

 The aminoglycoside antibiotics, such as streptomycin, are a class of protein synthesis inhibitors that are typically bactericidal. Streptomycin and other members of the class, such as kanamycin and neomycin, are produced by different species of *Streptomyces* bacteria. In nature, these substances protect the cell from other bacteria that may try to outcompete the *Streptomyces*. Releasing streptomycin and other antimicrobials protects the cell. As a chemical therapy, aminoglycosides bind to the small subunit of the bacterial ribosome. Normally, transfer RNAs (tRNAs) bring protein building blocks called amino acids to the ribosome that match the instructions on the mRNA, but when an aminoglycoside binds the ribosome, the tRNA brings an incorrect amino acid (Fig. 2). This causes a mutation in the protein sequence. Instead of being useful to the cell, the mutated protein is inserted into the cell’s plasma membrane. At the plasma membrane, harmful ions called hydroxyl radicals are produced, which damage the membrane and can cause the cell to breakdown. Tetracycline antimicrobials act in the same way as aminoglycosides, but are bacteriostatic instead of bactericidal.

 Macrolide and chloramphenicol antimicrobials all bind the large ribosomal subunit and stop the protein chain from being lengthened. These drugs are bacteriostatic and are typically only used when a patient is allergic to a more preferable substance such as penicillin or when a life-threatening infection does not respond to other antibiotics.

 Protein synthesis inhibitors have a good therapeutic index due to the structural differences between human and bacterial ribosomes, but they are less effective than cell wall synthesis inhibitors and generally only inhibit bacterial growth. They can also be toxic to the patient, so their use is closely monitored.

*Nucleic Acid Synthesis Inhibitors*

Another common target for antimicrobials is nucleic acid synthesis, or the production of the cell’s genetic material. Cells without genetic material such as DNA and RNA cannot produce the proteins necessary for survival. However, DNA, RNA, and the proteins needed to produce these molecules do not differ greatly between humans and bacteria.

The most commonly used nucleic acid synthesis inhibitors are quinolones and rifampin. Quinolones are synthetic, laboratory-made drugs. They combat infections by inhibiting DNA gyrase. DNA gyrase is needed during DNA replication. DNA is double-stranded, which means replication enzymes cannot access the bases unless the helicase enzyme unwinds the double-stranded DNA. However, unwinding the strands causes strain to develop ahead of the enzyme. Picture a piece of yarn that is falling apart: it becomes very loose at the top where it started to come apart, but the material moves to the bottom and becomes tightly packed. DNA works the same way, so the cell uses gyrase to relieve this so-called supercoiling to preserve the DNA. When quinolones inhibit gyrase, the DNA strand cannot be replicated properly.

Rifampin drugs inhibit the function of RNA polymerase. Even though the DNA is intact, if RNA polymerase enzymes cannot bind to the DNA strand no RNA will be produced, and RNA is necessary to produce proteins. Disrupting the replication-transcription-translation action of the cell is fatal.

Nucleic acid synthesis inhibitors are broad-spectrum antibiotics because they target an essential component of life: DNA and RNA. However, humans also have trillions of base pairs of DNA, which are not always distinguishable from pathogenic genetic material. As a result, the therapeutic index of quinolones and rifampin is lower than that of cell wall synthesis inhibitors or protein synthesis inhibitors.

*Antimetabolites*

The final major class of antimicrobials is the antimetabolites or metabolic antagonists. Antimetabolite drugs closely resemble molecules found in cells, especially substrates enzymes bind to. By outcompeting the substrate and binding to the enzyme, antimetabolites interrupt the metabolic pathway.

The best-known antimetabolites are sulfonamides (sulfa drugs) and trimethoprim. Sulfonamides are similar in structure to *p*-aminobenzoic acid (PABA). PABA is a cofactor (enzyme helper) that many enzymes responsible for folic acid synthesis require. Folic acid, or folate, is the foundation that nucleic acid bases and ATP, the energy currency of the cell, are built on. Without folate, the cell cannot make DNA or RNA. When sulfa drugs are present in the cell, they compete with PABA to bind to enzymes and cause the cell’s folate concentration to drop. Trimethoprim acts similarly and inhibits the synthesis of folate. Sulfonamides and trimethoprim are effective therapies because many microbes produce their own folate, but humans do not and must consume folate in their diets.

**Antimicrobial Resistance**

Antimicrobial resistance has captured headlines worldwide as more infections by “superbugs” are reported. Penicillin resistance appeared only years after it became commercially available. Some of this resistance is due to improper use of antimicrobials, while other resistance may be the result of positive mutations in bacteria.

Selective pressure is the idea that when a stressor is present some organisms will die while others will adapt and survive. When antimicrobials are prescribed to patients, the risk of exposing bacteria to antibiotics without killing them is present. Antimicrobials can be applied in too low a dose to kill or inhibit the pathogen. When this happens, the bacteria that survive (the “fittest”) will replicate and create a new generation of bacteria with the equipment to survive the same low doses of antimicrobial. The same outcome is possible when patients do not complete the regimen of prescribed antibiotics. Some people will take antibiotics until they feel healthy again and then stop because they think the infection is gone. However, bacteria that might have been eradicated by a few more doses of the drug can survive and proliferate.

Another scenario in antibiotic resistance is the acquisition of genes that will destroy the antibiotic. To combat attack by cell wall synthesis inhibitor antimicrobials, some pathogens have developed enzymes called beta-lactamases. These enzymes cleave the beta-lactam ring found in penicillins and cephalosporins. Once this active site of the antibiotic has been destroyed, the cell continues to synthesize the cell wall and is not destroyed.

Other bacteria have proteins called efflux pumps. Efflux pumps are active transport proteins that take toxic or waste substances within the cell and expel them into the cell’s environment. Once the antimicrobial is outside of the cell, it cannot inhibit protein or nucleic acid synthesis or perform any other bactericidal or bacteriostatic actions.

**The Future of Antimicrobials**

 Worldwide, pharmaceutical companies still work to design and produce new antibiotics with novel mechanisms to fight infections. In 2015, a coalition of German and American scientists published the discovery of a new antibiotic they call teixobactin. The team developed a method for isolating and growing bacteria in the soil that cannot be grown in the laboratory setting. From this soil sample, the researchers identified teixobactin, which acts by binding to lipid II, which is a precursor to the peptidoglycan of the bacterial cell wall, and binding to lipid III, the precursor to teichoic acid. Teichoic acid molecules are essential for determining cell shape, regulating cell division, and maintaining the integrity of the cell wall. By binding and blocking the actions of both lipid II and lipid III, teixobactin effectively obliterates the bacterial cell wall. In further experiments, the researchers could not identify any development of resistant to teixobactin in either *Staphylococcus aureus or* *Mycobacterium tuberculosis,* which are both notorious for developing antimicrobial resistance. Because both lipid II and lipid III are highly conserved, meaning they are present in virtually all bacteria, and essential to cell function, it is unlikely for bacteria to develop resistance to teixobactin, hence the audacity of calling teixobactin “resistance proof.”

 If teixobactin is less effective than hoped and resistant superbugs become more common, some people fear a return to a “pre-antibiotic era,” when many deaths were due to infections that are currently treatable. People imagine going back to a time when cuts and scratches become life threatening and little can be done to help an ill person. However, it must be noted that hygiene has improved greatly since Alexander Fleming unveiled penicillin in the midst of World War II. Understanding proper sanitation, the importance of avoiding contact with sick people, and even correct food handling procedures, can prevent hundreds of thousands of infections annually.

 While antibiotics are undeniably useful and convenient, they are not the only way to approach health care. If we want to continue to rely on antibiotics as heavily as we do now, it is essential to understand how to properly use these drugs. If antimicrobials someday become useless against superbugs, it will lead to a return to the common sense we all obey now: wash your hands, cook food thoroughly, and cover your mouth when you cough.

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